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STUDIES ON "BROWNING" ROOT ROT OF CEREALS

I. THE ASSOCIATION OF LAGENA RADICICOLA N.GEN.; N.SP., WITH ROOT INJURY OF WHEAT[1]

By T. C. Vanterpool[2] and G. A. Ledingham[3]

Abstract

A specific root rot of wheat and other cereals is described which is widespread over Saskatchewan, and occasions severe losses in some seasons. In early June, the outer leaves of the young plants become discolored, the number of tillers is reduced, growth delayed, and the yield considerably lessened. One of the chief diagnostic features is the presence of lesioned root tips containing oospores of the Pythium type. Although the disease is worst in a cool, wet spring, followed by warm, dry weather, the plants recover markedly when the remainder of the growing season is favorable. In preliminary work, "isolation strains" of various fungi from lesioned roots of field material collected in mid-summer failed to produce the disease under greenhouse methods of inoculation.

A fungus belonging to the lower Phycomycetes, hitherto not described, was found associated with rootlet injury of wheat, barley, rye and maize seedlings grown in Regina clay soil from infested fields of southern Saskatchewan. Its life-history, morphology and physiological characters are given in detail; it is believed to be an obligate parasite which, under conditions favorable to its development, is capable of causing definite injury to wheat. It is not considered one of the major causes of browning root rot, but in the section where it is common it is likely one of the contributing causes in some seasons. It is regarded as belonging to the Ancylistaceæ, close to the genus *Lagenidium*, but the erection of a new genus *Lagena* is recommended. The binomial *Lagena radicicola* has been assigned to the fungus.

More recently several "isolation strains" of Pythiaceous fungi have been secured from infected roots of wheat seedlings grown in soil collected from browning root-rot fields. A few of these in pot experiments in the greenhouse are shown to be definitely parasitic on the roots of wheat plants. There is some evidence that the particular root-rot concerned belongs to the *Pythium* root-rot complex type.

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Contribution from the Plant Pathological Laboratory of the University of Saskatchewan, Saskatoon, Canada, with financial assistance from the Saskatchewan Agricultural Research Foundation.

[2] *Assistant Professor of Plant Pathology, University of Saskatchewan.*

[3] *Research Assistant, University of Saskatchewan.*

The Disease

Introduction

For many years a root rot of wheat with characteristics distinct from the other better known root rots has been of great concern to the farmers of Saskatchewan and somewhat puzzling to the plant pathologists. Because of the brown color imparted to spots in the field by the discoloration of diseased seedlings in early June, the disease is becoming generally known as "browning" root rot. Its other chief diagnostic feature is the invariable presence of oospore-like bodies in lesioned roots. Attention was first directed to the disease in 1917 and the years immediately following, when it was particularly severe in the southern part of the province*; farmers in the northern parts also recall first encountering a serious outbreak about that time. The disease has varied in severity from year to year, frequently with recurrent outbreaks in the same field, and seems closely dependent on seasonal climatic conditions. In general, it appears to be most destructive on Regina Clay (14) soil, but is not confined to this soil type as its distribution is widespread throughout the wheat-growing area of Saskatchewan. More recently, partial and total crop failures have been reported on Silty Clay Loam and Silt Loam (15) soils of the Northeast. Both the hard spring wheats and the durums are susceptible to browning root rot, but no differences in varietal susceptibility have been noticed. During the 1929 season, it was definitely ascertained that oats and barley are attacked in the field. Rye was found to be susceptible when grown in pots containing soil from fields infested with browning root rot, but no case of the disease has been found on field collections of rye.

The disease appears to be more destructive on a wheat crop following a thoroughly tilled summer fallow than on stubble, or on fall or spring ploughed land. This fact, together with its occurrence in large areas in the field, and its widespread distribution, make it rank in economic importance as one of the most severe root-rot diseases of wheat in Saskatchewan, and warrant a systematic study of the underlying causes of the disease.

Literature Review

No extensive investigations on browning root rot appear to have been conducted hitherto. Before root rots and foot rots of cereals were closely studied in this province, browning root rot was thought to belong to the *Helminthosporium-Fusarium* group. Simmonds (19), in 1926, observed a rather extensive distribution of a Pythium-like fungus in the roots of cereal plants suffering from what was apparently browning root rot. The cereal crops showed symptoms of root rot early in the season; the plants recovered later, but growth was retarded. He does not mention specifically in which cereals he observed the Pythium-like fungus.

In 1922, *Pythium de Baryanum* Hesse was found associated with root blight of oats and barley in Denmark (11), the oats being severely attacked. To this fungus was attributed the cause of a seedling blight of wheat reported,

* *The authors are greatly indebted to Professor W. P. Fraser for this information, and also for general observations on the disease which have come to his attention over a period of years.*

in 1924, from Washington state (18). Beaumont has attributed to it the cause of damping off of oats in England; he also found evidence of delayed growth in plants that recovered (cited from 7).

A disease of barley, which in some respects appears to resemble browning root rot, was reported from Tunis (5); most of the crop in one field was sickly and failed to mature. Guyot found in the ends of the dead roots numerous round, thick-walled bodies which he identified as Pythium oospores, but he is of the opinion that they were not the cause of the disease. This writer also reports (12) the association of species of Pythium, etc., with the roots of chlorotic and yellowed winter wheat and oat plants from spots in fields near Paris where water had been lying for some time.

Subramaniam (20) described from India a disease of wheat caused by *Pythium graminicolum* n.sp. Oospores were found in rotten portions of the root and leaf-sheaths, but in contradistinction to browning root rot the collar or crown was also necrotic and brown, the discoloration extending to the first node.

A fungus isolated from the root of an oat plant in Wisconsin state was described by Drechsler (7) under the binomial *Aphanomyces camptostylus* n. sp. but its pathogenicity on oats was not tested. Its oospore is not unlike the *Pythium* type commonly found in the roots of cereals attacked with browning root rot.

In inoculation experiments conducted in sterilized soil Edgerton and his co-workers (8) have found that wheat is very susceptible, and oats slightly less so, to their sugar-cane *Pythium*. The type of injury produced on the roots of the wheat and oat plants was very similar to that produced on cane.

Symptoms

The presence of the disease in a field is first noticed in early June when the seedlings are a few inches high. Usually, spots of various sizes and shapes in the field are seen to turn yellow and then brown, giving a general appearance of drought and starvation symptoms. The discoloration is caused by the death of the outer leaves.

According to present observations the plants are not killed outright, but recover, and give a fair yield when the remainder of the growing season is favorable. This recovery is remarkably striking, especially when unusual rainfall follows the appearance of early symptoms. The rainfall tends to mask the symptoms. Farmers who, in June, may have contemplated ploughing up attacked fields, are pleased to find by early August that the yield will probably be only a few bushels below average. Ordinarily, however, the affected plants are reduced to one or two tillers, stunted, and are late in maturing (Fig. 1, *A*). This condition gives the weeds a good opportunity for growth and hence it is sometimes an easy matter to locate from the roadway, by the excessive growth of weeds, what was earlier in the season a "browning patch". The plants are not now noticeably lighter in color than healthy plants; and, in fact, towards harvest, may actually be greener.

When diseased plants are carefully pulled up and the soil shaken or, better washed off the roots, the majority of root tips are seen to be distinctly brown and necrotic (Fig. 1, *B*). The ends of the secondary or crown roots are most strikingly attacked. Not infrequently, brown lesions are found scattered along the main roots. The subcrown internode, crown and leaf-sheaths are invariably free from blemishes, that is, no foot rot is produced (20). Taken as a whole, the root system is thin and there is a scarcity of fine laterals and root hairs, which doubtless accounts for the small amount of adhering soil.

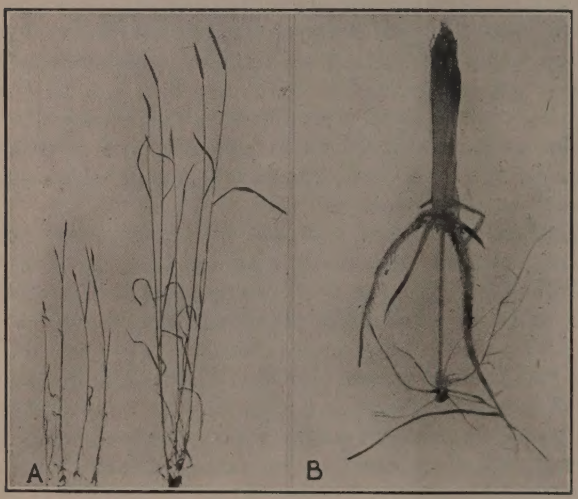


FIG. 1. *A, Left, four single tillered, stunted plants from a "browning" spot; right, two healthy plants from the same field. B, Portion of a diseased plant showing the characteristic lesioning of the root tips, and the clean crown and subcrown internode.*

When a necrotic root tip is teased apart with dissecting needles and examined microscopically, brown, smooth-walled, oospore-like bodies (Fig. 2), are constantly found to be present; these are confined mainly to the cortical, epidermal and root hair cells. However, it is not uncommon to find these bodies in the vascular bundles, thereby showing that the stele is also invaded. The oospores occur singly or aggregated in one cell. No hypertrophy of host tissue is discernible. At this stage, the presence of hyphæ or hyphal remains can seldom be detected in the diseased roots even after careful searching and staining; if found, however, no connexion with the oospores can be traced.

ECOLOGY

The disease appears to be most destructive in seasons with a cold, wet spring followed by an abrupt spell of drought and warmer weather, the "browning" symptoms then appearing suddenly. Under such conditions the damaged root system of the wheat plant is unable to meet the demands made upon it by increased transpiration; this results in the outer leaves dying and as a consequence browning symptoms appearing above ground. Should this occur when the wheat plants are tillering, as often happens, it would doubtless account for the reduction in the number of tillers in attacked plants.

The conditions outlined above seem to be the chief meteorological predisposing factors; but the consistent presence of the oospore-like bodies in roots from widely separated localities is evidence which favors the attack of a fungous parasite as one of the chief contributing causes.

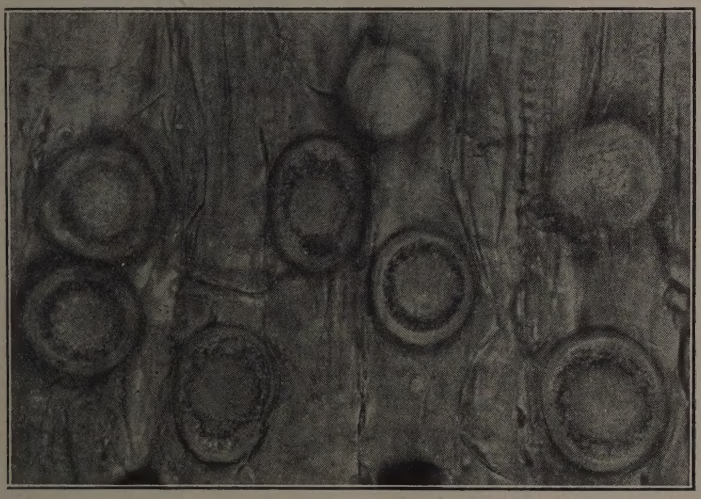


FIG. 2. *Oospore-like bodies commonly found associated with lesioned roots in "browning" root rot.* × 700.

Browning root rot has been found under all types of cultural practices in vogue in the province; but, as already mentioned, it appears to be worst on early ploughed, thoroughly cultivated, summer-fallowed land. It is sometimes as severe on land which has been under cultivation for only a few years as on land 20 or 30 years under cultivation. There is at present no reliable evidence at hand to show that the disease increases on land grown to wheat only; recurrent outbreaks do occur on such land, but it is uncertain whether they are increasing in severity. A wheat-oats-summer-fallow rotation does not seem to decrease this root rot. Fields including sweet clover or alfalfa in a rotation over a period of years have been found to contain traces of the root rot. In some localities applications of farmyard manure and of well-rotted wheat straw, as found in old "straw bottoms", inhibit the disease to some slight extent. To date, no symptoms of browning root rot have been observed in wheat grown on virgin prairie soil, but no extensive search has been conducted to verify this.

Work has been started in an attempt to ascertain whether or not there is any correlation between soil reaction and the incidence of the disease. The pH values of soils from several severely infested fields from widespread localities ranged from pH 7.0 to pH 8.0, but more data are needed before definite conclusions can be drawn.

Preliminary Studies

To test the truth or fallacy of the parasitic nature of the disease, attempts to isolate the causal organism by tissue culture and by germination of the oospores were accordingly made. Use was made of diseased wheat plants sent in by farmers during June and the early part of July, 1928. Later, roots of diseased plants collected during late July and early August on a root-rot survey of the central and northern sections, were used in the isolation work.

In the isolations from diseased tissue, it was soon found that if aqueous mercuric chloride (1:1000) was used as the sterilizing agent in the usual manner, if only for a short time, no fungal growth could be obtained from the tissue platings. The procedure finally adopted was the following: the young diseased plants as soon as received were thoroughly washed in running water. The root-tip lesions were then clipped off with sterilized scissors and placed in sterile water in a small flask, where they were agitated by continuous vigorous bubbling with sterile air; the wash water was repeatedly changed. Under aseptic conditions the washed tissue was transferred to Petri plates of potato dextrose, oatmeal, cornmeal and water agars, half of which had been slightly acidified to keep down bacterial contamination. On the acidified media only *Fusarium* species were obtained from the early platings; but on the non-acidified media *Pythium* forms could occasionally be found growing out of the tissue. Where both a *Fusarium* and a *Pythium* were growing out from the same diseased piece, the *Fusarium* would invariably outgrow the *Pythium*, thus making isolation of the latter almost impossible. Occasional species of *Alternaria*, *Helminthosporium*, and a *Mucor* which formed an abundance of chlamydospores on cornmeal agar, were also isolated. Seven "isolation strains" of chlamydospore-forming *Fusaria*, two of *Pythium* forms, and the chlamydospore-forming *Mucor*, were selected for pathogenicity studies on wheat; that is, those forms which might account for the oospore-like bodies in the lesioned roots.

All possible attempts to germinate the oospore-like bodies were made, using both spores in mass in well crushed tissue, and single spores. Hanging-drop cultures in sterile tap water, sterile distilled water, soil extract, and various plant decoctions, were prepared and incubated at different temperatures; repeated changes in some of the water cultures were tried as well as changes from the soil extract and decoctions to water and *vice versa*. Various concentrations of hydrogen peroxide, subjection to pressure and to ultra-violet light, were also tried. Both oospores formed during the current season and over-wintered oospores were used in these tests, but in no single instance was germination obtained. Germination attempts on various solid media also proved futile.

The *Fusarium* cultures obtained from the tissue isolations produced abundant chlamydospores on cornmeal and oatmeal agars; the *Pythium* cultures grew well and produced abundant conidia and sporangia, but no oogonia or oospores were ever observed. The *Fusaria* and the *Mucor* were grown on sterilized, moistened, crushed oat hulls for two or three weeks; 20 gm. of this

inoculum was then mixed thoroughly in five-inch pots with the top three inches of soil previously sterilized in an autoclave, and the mixture sown with surface sterilized wheat. The *Pythium* cultures were grown on a sand-cornmeal mixture (16) for about three weeks; 40 gm. of this inoculum was used to each five-inch pot. In the control pots, sterilized oat hulls were mixed with the sterilized potted soil as a check on the *Fusaria* and *Mucor* inoculations, and sterilized sand-cornmeal was used in a similar manner as a check on the *Pythium* inoculations. All of these pots were kept in the greenhouse with a temperature range from about 50° F. to 75° F. Pure high grade seed of Marquis, Garnet, and Mindum (Durum) were used in these tests.

The greenhouse pot tests were also supplemented by tests for parasitism on wheat growing on agar plates.

Of the various isolation strains tested, three of the *Fusaria* showed slight parasitic tendencies. Seedlings in pots inoculated with these strains, when a few weeks old, began to look sickly and turn yellow gradually. A microscopic examination of the underground parts revealed several brown lesions scattered along the roots, and only occasionally a lesioned root tip. When examined microscopically the lesions were seen to be filled with hyaline, septate mycelium and typical *Fusarium* chlamydospores—a picture very unlike that of a wheat root attacked with browning root rot.

In the pots inoculated with the *Pythium* strains and the *Mucor*, a root tip would at times be found which showed the presence of non-septate, hyaline mycelium and occasionally more or less spherical bodies, conidia or sporangia in the *Pythium* pots, and chlamydospores in the *Mucor* pots. The roots, however, showed no typical brown lesioning; neither were the bodies typically oospore-like as in true browning. On the whole, there was no appreciable pathogenicity shown.

From these preliminary studies it must be concluded that under the conditions of the experiments the particular strains of fungi isolated from root lesions of diseased wheat plants collected about the middle of the growing season are not the cause of the disease.

However, as described later in this paper, it was not until isolations were made from the roots of wheat seedlings grown in pots of soil from root rot infested fields that Pythium-like forms were obtained which produced abundant oospores in culture and showed definite parasitism on wheat seedlings under greenhouse conditions of experimentation.

The Association of Lagena Radicicola n. gen.; n. sp., with root tips of wheat grown in infested soil

Soil from Infested Fields as Source of Inoculum

In January, 1929, some soil was secured from a field near Regina which had borne a summer fallow wheat crop badly infested with browning root rot the previous season. Surface sterilized wheat was sown in this soil in five-inch pots in the greenhouse. Two pots of the soil were steam-sterilized and used as controls.

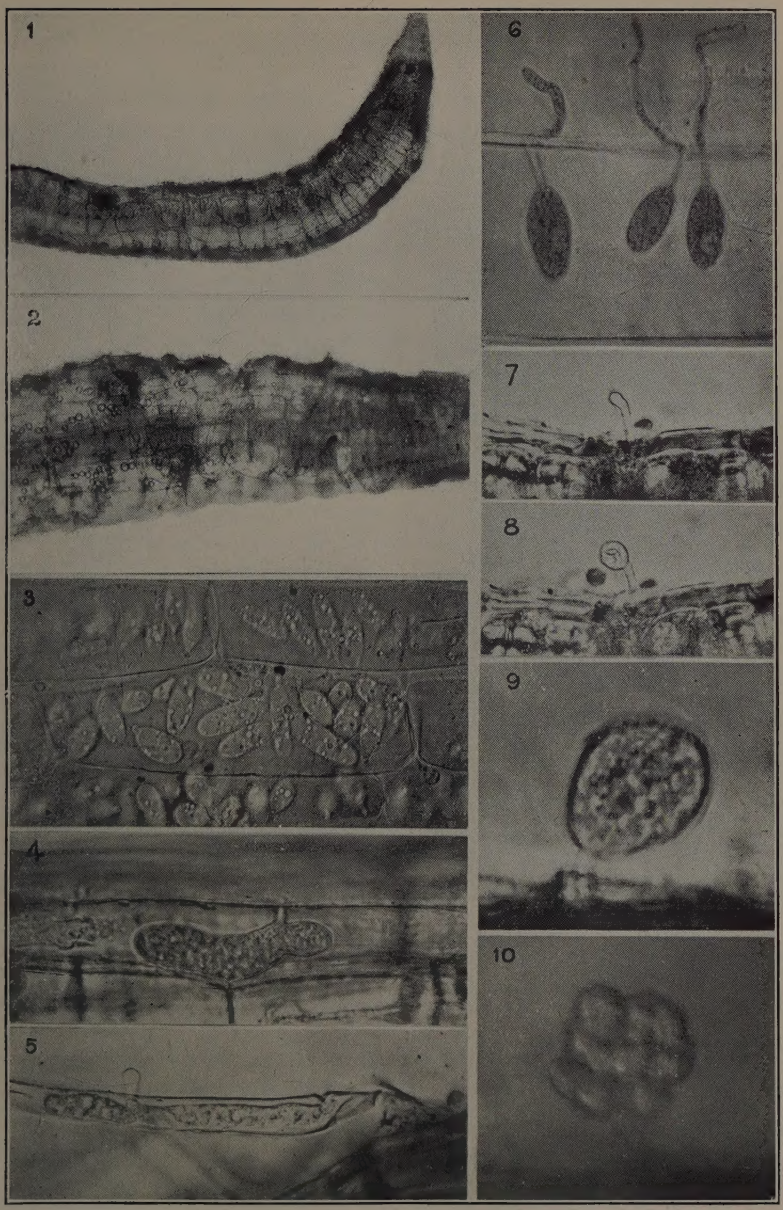
When the seedlings were about three inches high disease symptoms, indistinguishable from those of browning root rot in the field, developed in the pots of unsterilized soil. At this stage, 17-21 days, no lesioning was in evidence on the roots, but an examination of root and rootlet tips revealed a few bodies quite similar to the oospore-like bodies in browning root rot, together with numerous one-celled, sac-shaped sporangia, attached by beaks to the walls of the epidermal and outer cortical cells of the host (Plate I–1 to 4). No mycelium connected either with the sporangia or the spherical spores could be observed. A large percentage of affected rootlet tips was characteristically curved as shown in Plate I–1. The fungus showed a decided preference for cells about the growing point, and by interfering with growth activities probably accounted for the curvature just referred to. Otherwise, there was no hypertrophy and apparently no attempt on the part of the host to react against the invading organism.

FIG. 3. *Pots A and C contain Marquis and Mindum respectively, in Regina Clay soil from a "browning" root rot field. B, contains Marquis grown in autoclaved soil. All plants are five weeks old.*

Examinations of successively older plants showed a relative increase in the number of thick-walled spores and a decrease in the sac-shaped sporangia, until finally, in plants about five weeks and older, only the thick-walled spores could be found. Such plants possessed a thin root system with yellowish brown lesioning of the root tips. Fig. 3 shows the plants in this series at five weeks, and Fig. 4 at maturity.

Compared with such a picture the plants in the control pots of sterilized soil were healthy looking, green and taller, with a thick, white root system with copious fine rootlets. No fungus was in evidence in the roots. It is not surprising that under such artificial conditions plants in soil previously sterilized in an autoclave should show better growth than those in the unsterilized field soil. From the standpoint of the writers, two facts may be inferred: either, (a) that the treatment in the autoclave so changed the chemical composition of the soil as to remove substances deleterious to the growth of wheat plants, or made available substances conducive to better growth; or (b), that one or more organisms parasitic to the wheat plants were killed by the sterilization.

Preliminary attempts to isolate the fungus were not successful, and in the meantime, as the absence of mycelium strongly indicated it to be an obligate parasite, a close study of this fungus and its relations to wheat and other cereals in living root tips was accordingly undertaken.



Lagena radicicola

FIG. 1. *An infected root tip of wheat characteristically curved, showing both thalli (zoosporangia or cænogametangia) and resting spores.* × 70. FIG. 2. *An older root tip than that shown in Fig. 1; resting spores only are to be seen.* × 100. FIG. 3. *An epidermal root cell containing numerous thalli of the fungus, resulting from multiple infection. The root had been mounted in water on a glass slide for 24 hr when the photomicrograph was taken.* × 420. FIG. 4. *A single mature individual in an epidermal host cell.* × 400. FIG. 5. *A single individual in a root hair cell with its tube of discharge already well formed. The condition of the hyaline cap indicates that the contents of the sporangium will be discharged into a vesicle in a very short time.* × 600. FIG. 6. *Three sporangia with abnormally long exit tubes. Root tip was killed and stained in lacto-phenol-acid fuchsin.* × 900. FIG. 7. *A tube of discharge immediately before vesicle formation.* × 280. FIG. 8. *The hyaline cap of the discharge tube seen in Fig. 7 has blown out into a gelatinous vesicle.* × 280. FIG. 9. *An early stage in the formation of a vesicle, the gelatinous envelope being still visible. The asymmetry of the undifferentiated protoplasmic contents is due to its contact with the cover glass.* × 1000. FIG. 10. *An aggregation of moving zoospores, still retained by the gelatinous envelope at the mouth of the discharge tube, about one minute before they dispersed.* × 900.

Method of Studying the Wheat Fungus

Plants were grown in pots as described above, and removed neatly from the pots with root system and adhering soil intact, when an examination of the roots was to be made; this was done by inverting a pot and tapping carefully on a wooden bench. Any amount of root system required could now be removed for study, but all that was found necessary was to clip off with scissors some of the finer roots and rootlets growing on the outside of the soil, and then replace the practically undisturbed soil and plants in the pot for further study as required. All the plants were kept well watered. The roots to be examined were washed in running tap water to remove adhering soil particles; usually this would not be a difficult matter as the roots growing on the outside of the soil, where it comes in contact with the inside of the pot, are relatively free from particles, and only occasionally had a camel's hair brush to be used to aid their removal.

Fig. 4. *Left, plants grown in Regina Clay soil from a "browning" root rot field. Right, plants of the same age grown in autoclaved soil.*

Rootlet tips a few millimetres long were mounted in water on microscope slides with thin cover glasses. When a prolonged study was necessary, the rootlet tips were washed in sterile water and mounted in either sterile tap or distilled water, the slides being kept in a moist chamber at temperatures not exceeding room temperature. During continuous microscopic examinations water had frequently to be added to replace that lost by evaporation.

Sowings of wheat in pots of infested soil were made every two or three weeks beginning early in February, so that fresh material would almost constantly be available for observation. With a few short interruptions our observations continued until May.

A study was also made of the fungus in stained sections. Flemming's strong solution diluted one-half with water, chromo-acetic solution, and Bouin's fluid were used as fixatives. Various stains were tried but the only one found to give fair cytological detail was iron-alum hæmatoxylin. The other stained preparations merely confirmed our observations on the morphology made under living conditions. Excellent differentiation of fungous and host tissue was readily obtained by placing infected roots directly into lacto-phenol-cotton blue or lacto-phenol-acid fuchsin, and afterwards mounting in the lacto-phenol solution.

Life History and Morphology

The zoospore and infection

The entrance of the fungus into the host tissue is effected by means of free-swimming zoospores, which under certain conditions abound in the water about the roots of wheat seedlings.

Fig. 5. *Lagena radicicola. A, Semi-diagrammatic representation of infection of a root hair cell of wheat, resulting in the formation of a single thallus e, which is a potential zoosporangium or a potential cœnogametangium. B, Two mature thalli, a and b, and an empty sporangial sac c, in a host cell. The attachment of the thallus a to the cell wall is seen in surface view as two concentric rings; in b, the collar is seen in side view. Compiled from camera lucida drawings.* × 1200.

The zoospore, immediately after discharge, is bean-shaped, about 7 μ wide by 11μ long, with two flagella arising from the lateral depression (Fig. 5; *A*, *f*). Its contents are coarsely granular with several dark granules and two or three small vacuoles representing lighter areas. Usually, the swimming movements are quite rapid with characteristic sweeping arcs and an occasional shuffling interspersed between direct forward movements. Not infrequently, a zoospore is observed travelling forward in a straight line, more or less, with one flagellum trailing and as far as can be judged, with the other in rapid motion. Much time has been spent in continuous observation of the zoospores but no evidence of any conjugation has been obtained. On one occasion in a 24-hour preparation on a slide, two zoospores which had lost their flagella were seen to divide each into two, the resulting daughter spores also assuming a spherical form; the ultimate fate of these was not observed. Why this should have occurred remains unexplained. It seems probable that it is not a normal condition. As far as the present observations go it seems that the zoospore swims actively

in the water about the root for several minutes; its movements then become slower and slower until finally its flagella disappear and it rounds up into a ball coming to rest on the surface of a root hair, an epidermal cell or the glass slide (Fig. 5; *A*, *a*). An amœboid movement previous to the final rounding up, and reminiscent of a similar phenomenon as described by Butler (3) for Chytrid zoospores attacking *Pythium* species, has occasionally been observed. The encysted zoospore is slightly smaller than the motile one, averaging 6μ in diameter. The ultimate fate of those on the glass slide is unknown; doubtless after a time they break up as described by Butler (3) for the zoospores of certain Chytrids, and by Kusano (17) for the zoospores of *Olpidium viciæ* Kus. On not more than three or four occasions during the whole period of study, some of these encysted zoospores in water mounts were observed to germinate by means of short germ tubes. When entrance into the host is about to occur the encysted zoospore on the surface of the root hair or epidermal cells puts out an infection tube which penetrates the thin cell wall, the zoospore at the same time becoming slightly raised above the surface of the host (Fig. 5; *A*,*b*). The contents of the encysted spore gradually move into the infection tube which, after extending well into the lumen of the host cell, begins to enlarge into a germ-sphere, becoming typically sac-shaped in the larger epidermal cells where its development is not hindered, and considerably more elongate in the narrower root hair cells (Fig. 5, *A*,*e*, and *B*). The spore shell persists for a short time on the surface of the host and then ruptures.

Thus the contents of the zoospore have developed directly into a mature thallus which, even from its very earliest stages, is apparently always attached to the cell wall by a neck which fits into a thickened collar (Fig. 5, *B*,*b*). This collar is presumably an outgrowth from the host cell wall, for if slight pressure is applied to the sporangium, its tapering neck will come away intact, leaving the collar attached to the cell wall of the host as seen in side view in Plate II–7. In surface view, the collar appears as two concentric rings (Fig. 5, *B*,*a;* and Plate II–6). As far as we have been able to ascertain, at no time after entrance is the zoospore free within the living host cell. This explains why the sporangia are invariably attached to the outer cell walls, why the host nucleus is seldom in close association with the individual thallus, and why cleavage of the individual during growth, as occurs in certain Woroninaceæ, has never been observed. An aggregation of sporangia in one cell may be the result of multiple infection, or the discharge of zoospores internally, as described later.

Thallus Characters

The protoplasm of the parasite is always distinguishable from that of the host. The thallus soon becomes multinucleate, its contents during its whole period of development are usually highly refringent and vacuolate until shortly before germination when the protoplasm becomes granular and homogeneous, and finally, just preceding zoospore-discharge, it collects into zoospore initials. The size of the mature thallus varies considerably depending chiefly

on the number present in each cell (Plate I–3 to 6). A single individual may sometimes completely fill the host cell, when it may attain considerable length or become more or less hook-shaped; but where there are several individuals in one cell the competition for both food and space limits their development.

The mature thalli may do either of two things: (a) they may germinate producing zoospores, thereby functioning as zoosporangia or, (b) they may conjugate in pairs producing thick-walled sexual resting spores (oospores), in which case they may be considered as cœnogametangia. There is nothing to indicate whether a mature thallus will function as a zoosporangium or as a cœnogametangium. The germination of the zoosporangium will first be described in detail.

Asexual reproduction

A single exit or emission tube grows out from the mature sporangium as an elongation of the neck into the surrounding water (Fig. 6, *A*). More than one discharge tube to each sporangium has not been seen, and this is what should be expected. There is a slight constriction in the tube where it passes through

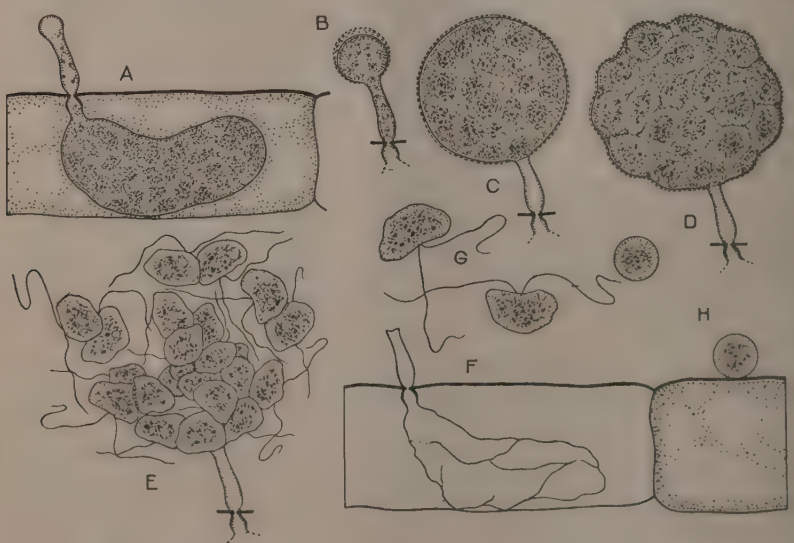


FIG. 6. *Lagena radicicola. Stages in the germination of a sporangium. A, a mature sporangium with exit tube. B to E, its contents are discharged into a vesicle where the zoospores are formed and liberated. F, the remains of the empty sporangial sac with exit tube. E, free-swimming zoospores. H, an encysted zoospore on surface of a host cell. Compiled from camera lucida drawings.* × 1200.

the cell wall. It is about 4 μ wide and usually varies from 10 μ to 15 μ in length. Exit tubes over 20 μ in length are occasionally observed (Plate I–6), but these are so infrequent that they are probably not normal. At first the apex of the exit tube is no wider than the tube itself, but when growth in length

ceases the apex enlarges slightly becoming symmetrically rounded, and the cell wall becomes characteristically refractive and gelatinous. The protoplasm in the tube remains clear, but there is a continual slow streaming of a few refringent dark granules through it. This is in striking contrast to the protoplasm of the sporangium itself which is darkly granular and heaped up into zoospore initials. Just before the discharge of the contents of the sporangium the protoplasm in the sub-apicular portion of the tube becomes exceptionally clear, the dark granules which continue to stream slowly in the tube below do not enter the sub-apex; whether or not this is a sub-apicular vacuole, as described by Butler (3) for *Pythium rostratum* Butler, could not be ascertained definitely. However, the immediate changes which follow, resulting in the production and liberation of zoospores, correspond closely with Butler's description of the same phenomenon in *P. rostratum*. That is, the cap of the exit tube blows up into a bladder into which the protoplasm from the sporangium rapidly flows. The gelatinous bladder is at first hyaline and distinctly visible, there being a definite space between it and the advancing protoplasm (Plate I–9). Later, the protoplasm fills the bladder faster than it grows, so that the intervening space gradually diminishes until finally the protoplasm completely fills the bladder (Fig. 6, *C*). The envelope can now no longer be recognized, but its surface is very highly refractive. The time taken for the discharge of the sporangial contents into the vesicle varies from about one to four minutes depending on the size of the sporangium. Towards the end, the rate of flow is considerably lessened, and there is a tendency for it to be jerky. During the period of flow the dark granules of the viscous protoplasm are continually tumbling over one another, thereby giving it a homogeneous appearance which in no way indicates the presence of zoospore initials. This rearrangement of granules continues in the vesicle and within a minute or two of the completion of protoplasmic discharge the demarcation of zoospores becomes apparent, corrugations appear on the surface and movement of the whole mass, with the empty efferent tube acting as a pivot, begins (Fig. 6,*D*). As the delimitation of the individual zoospores proceeds, this oscillating motion is gradually replaced by the individual motion of each zoospore even before there is any sign of flagella formation. There is a gradual enlargement of the whole mass which allows of greater freedom of the individual zoospores whose shuffling movements become ever more and more pronounced. The lashing of flagella is soon observed and a rearrangement of the zoospores within the mass usually occurs. The motion of those on the outside gives the impression that there is an invisible envelope restraining their action and preventing their escape; but such a membrane is not discernible to the aided eye although the authors have observed the discharge of a score or more of zoosporangia. Under similar optical conditions the vesicle membrane of a *Pythium sp.* immediately preceding zoospore escape was distinctly visible. Finally, the vigorous movement of the zoospores appears to burst this invisible enclosing membrane as two or three first dart away, the remainder immediately following (Fig. 6, *E*). The remains of the empty sporangium and exit tube are shown at *F*, Fig. 6. The time taken from the beginning of streaming to zoospore liberation is 15 to

17 minutes at room temperature. It frequently happens that after zoospore discharge, batches of two or three remain held together presumably by their flagella, but a violent tugging continues and their separation is soon complete. The number of zoospores from each sporangium varies from a few to over 50. After coming to rest on the surface of the host, they can again initiate infection and thus complete the asexual cycle in the life-history of the organism (Fig. 6,*H*).

The temporary sporangium within the host may therefore be considered as the presporangium and the vesicle as the zoosporangium proper.

Quite frequently, but apparently under a specific set of conditions, zoospores which have been discharged internally, are observed swimming actively within a dead cortical or epidermal host cell. As in other observed forms, under these confined conditions the zoospores remain motile for a much longer period than those liberated outside the host cell. In one instance zoospores in a host cell were observed to retain their motility for over six hours at about 15° C.

As already described the individual protoplast which develops into the presporangium evidently always remains attached to the cell wall at the point of infection; at this point, at maturity, an exit tube emerges and allows of the discharge of the contents of the presporangium. Hence there must be some other means of zoospore-discharge to account for the presence of free-swimming zoospores within a host cell, at least in the first instance. Two possibilities suggest themselves: (a) the emptying of the contents of a presporangium into the same cell through a tube of discharge, and (b) the bursting of the thin presporangial wall, brought about perhaps by the sudden addition of fresh water to the roots. The presence of an empty presporangial sac in the same cell with active zoospores is not in itself sufficient evidence to prove that the second possibility (b) is the correct explanation, as its contents may have been discharged extramatrically. Once free within the host cell, these zoospores doubtless penetrate the wall of the confining host by a germ tube which enters one of the cells immediately surrounding and there develops into a new individual in the usual manner. It might be that the zoospores after encysting on the wall of the confining host cell become attached to it and develop directly into mature individuals, thus never leaving the cell.

Sexual reproduction

Sexual fusion may take place wherever there are two or more individuals in the same cell. One of these, the male, puts out a conjugation tube about 8 μ in diameter, which fuses with another individual, the female (Fig. 7; and Plate II–3 to 5). The conjugation tube or antheridium enlarges slightly where it comes in contact with the female, but the actual opening at the point of contact is very small. If conjugation occurs between two individuals which are almost touching, the antheridium is merely a swelling of the male at the point of contact with the female. Should the conjugating individuals be at opposite ends of an oblong cell, the antheridial tube will extend right across the lumen of the host cell. Conjugation between individuals in adjoining cells

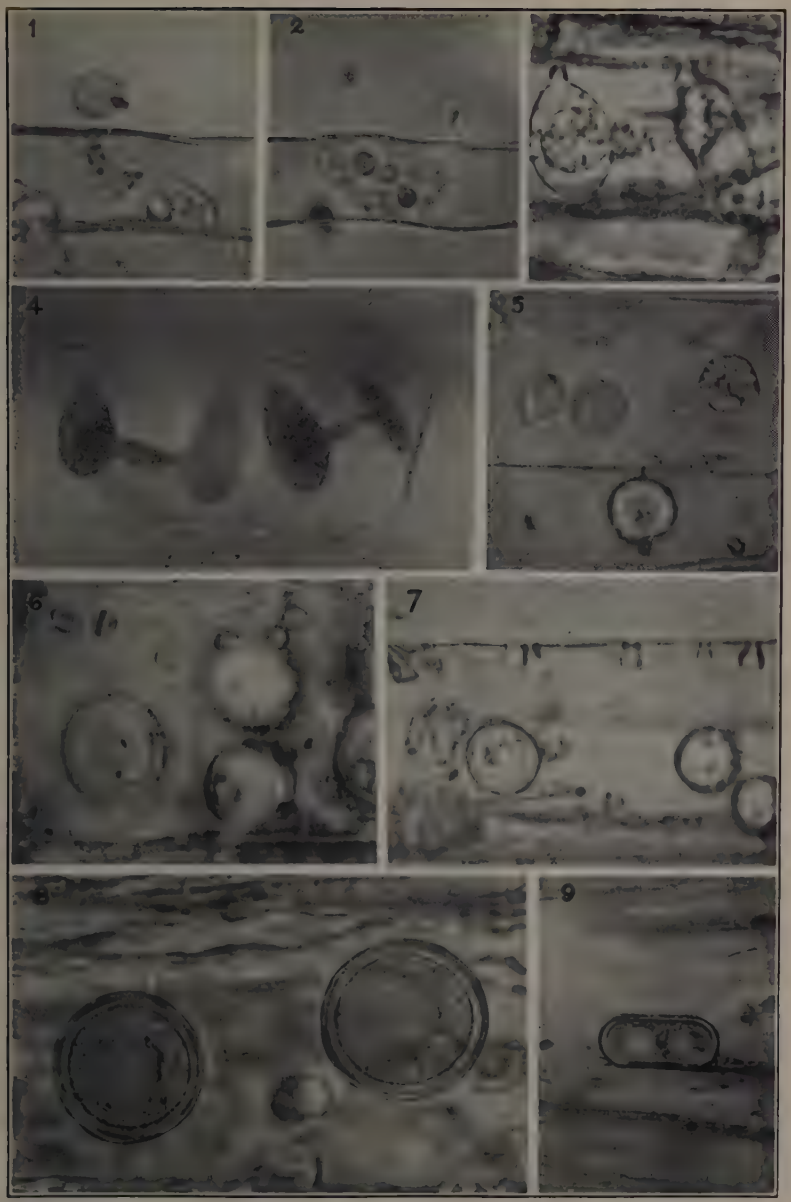


FIG. 1 AND 2. *Infection of root hairs of wheat grown in sterile water in a small flask. The zoospore cysts, slightly raised above the surface of the host, have not yet ruptured. The infection tube has already enlarged considerably. Fig. 1,* × 1400; *Fig. 2,* × 1350. FIG. 3. *At the top, right; the male thallus on the left has almost completely emptied its contents into the female, where the combined plasmas have contracted and rounded off. A thick cell wall is being laid down around this mass. The antheridial tube, not very distinct in the photograph, is quite short. In the lower cell, two other conjugating thalli are to be seen, slightly out of focus.* × 900. FIG. 4. *Two pairs of conjugating thalli in a host cell. In each pair the female thallus is on the left and the male on the right. The contents of the male thalli have not yet begun to pass into the female thalli. The root tip was killed and stained in lacto-phenol-cotton blue.* × 900. FIG. 5. *Two conjugating thalli, the female with its spherical oogonium on the left, and the male on the right. Note the characteristically club-shaped antheridial tube.* × 900. FIG. 6. *The collars or vents where the thalli were attached to the cell wall of the host are seen in surface view as concentric rings. Sexual resting spores (oospores) are seen in the cell beneath. Providing all the thalli have conjugated in pairs there will be two vents to each oospore.* × 900. FIG. 7. *The collars or vents are shown in side view attached to the host cell wall. The empty thallus membranes have disentegrated.* × 650. FIG. 8. *Mature oospores in host cell.* × 1400. FIG 9. *An oblong oospore with two central globules.* × 420.

has never been observed. The male thallus is not consistently smaller than the female, as in the genera *Olpidiopsis* and *Pseudolpidium* (2, 10); occasionally it is actually larger. During fertilization the protoplasm of both individuals congregates into droplets, the contents of the male pass very

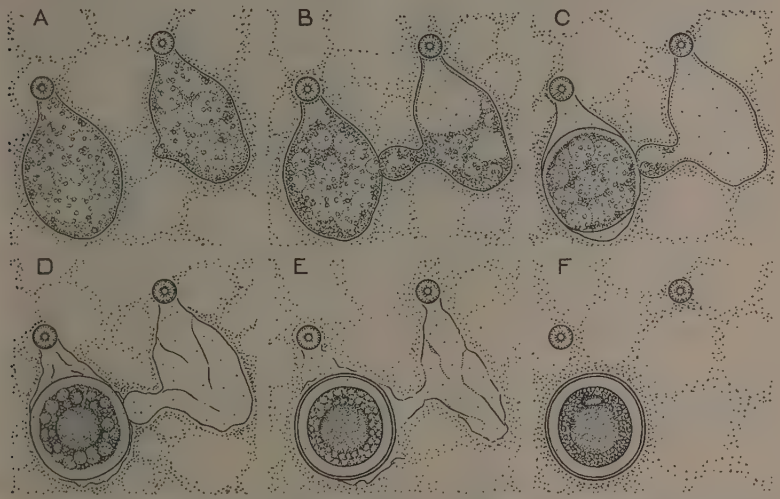


FIG. 7. *Lagena radicicola. Semi-diagrammatic representation of conjugation of two cœnogametangia resulting in the formation of an oöspore. In surface views the points of attachment of the conjugants to the cell wall of the host appear as concentric circles. Compiled from camera lucida drawings.* × 1200.

slowly along the antheridial tube into the female sac, where the combined plasmas contract and assume a spherical form. While fertilization is in progress there is no visible evidence of an oosphere within the oogonial sac. A thick cell wall is now formed about this mass which further diminishes in size and lays down an inner wall (Fig. 7,*E*). During the maturation of the sexually-formed spore the protoplasmic droplets gradually decrease in number, but the remaining ones are proportionally larger, until finally there is one large central globule and a flattened refringent body embedded in finely granular protoplasm within the smooth, double, cell wall. The oospores vary from spherical to oval, but occasionally an oblong one is found which possesses two large central globules instead of one. The cytological details involved in the sexual process remain to be worked out. All that is definitely known is that the two conjugants are multinucleate. The authors have not succeeded in germinating the oospores.

The empty remains of the male and female thalli as well as the antheridial tube, persist only for a short time after fertilization is complete. Later, no trace of them can be found; there remains only the oospore, and the collars of the two conjugants attached to the cell wall (Fig. 7,*F*). There is no evidence of the remains of the male persisting as an "appendiculate cell" on the oospore as in the genus *Olpidiopsis*.

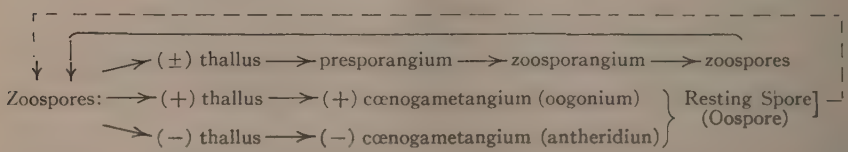
Several instances of apparent triple fusion have been observed, but the location of the organism within the host cells made accurate observation difficult. As far as could be judged, there were two antheridial tubes, from two separate male thalli, attached to a female cell in which an oospore was forming. It seemed as if the contents of both male cells were being discharged into the female. This is similar to what sometimes happens in *Pseudolpidium saprolegniæ* (10). With the present organism more observations will be necessary to settle this point. However, it is possible that triple fusion may account for the oospores with two central globules (Plate II-9).

It is not known definitely if a single thallus can develop parthenogenetically into a thick-walled oospore in the absence of another (male) thallus. Single oospores, frequently found some distance from groups of cells heavily infested with the fungus, give some indication that this might be possible.

As is to be expected from the great variation in size of the mature individual thalli, as previously described, the oospores show a wide range in size.

Life-history diagram

Reasoning by analogy of known forms we may assume that the oospores germinate either directly or indirectly producing zoospores. With this fact only unverified, the following tentative life-history diagram may be constructed:



Host Range and Relationships

The experiment described below is typical of very many others conducted to ascertain the relationships of the organism to various host plants. The failure to grow the organism on culture media necessitated this type of investigation.

Pot Experiments

On March 7, 1929, three five-inch pots of soil from a browning root-rot field were sown with surface sterilized Marquis wheat, and three with Mindum (Durum). Two pots of the soil sterilized in the autoclave were sown to Marquis and Mindum respectively, to serve as controls. The pots were kept on the greenhouse bench.

On March 22, when the seedlings were three inches high their roots were examined in the manner already described. Temporary sporangia only were found in about 25% of the root tips examined. During the next few days the percentage of infected root tips increased, and on March 25, conjugation of temporary sporangia (cœnogametangia) and the formation of oospores were

first observed. Subsequently, the number of temporary sporangia decreased and that of oospores increased, so that finally when the plants were over two months old, only mature oospores could be found in lesioned root tips. As might be expected, there are at this stage practically no fine rootlet tips filled with oospores; but it is most probable that owing to their delicacy and the intensity of infection, they have completely disintegrated. Although the fungus does not enter the stele, it is frequently found in the meristematic tissue of rootlet tips and doubtless inhibits, and sometimes even prevents, further growth in length. The plants in the unsterilized soil soon assume a sickly appearance; their outer leaves become discolored and growth is delayed. This results in fewer tillers per plant, later maturity and considerably lower yield compared with the plants in the unsterilized soil.

Under these experimental conditions Reward, Garnet and Mindum wheat, Keystone barley, and Prolific rye, are readily attacked by the parasite, the symptoms being as described above for Marquis, and no difference in the degree of susceptibility is distinctly discernible. To ascertain this with accuracy, soil temperature and soil moisture studies are necessary. Arrangements are being made to conduct such studies.

The finer roots of maize were occasionally found to be attacked, but Victory oats, flax, *Agropyron tenerum* Vasey, *A. spicatum* (Pursh.) Scribn. & Sm., *Bromus inermis* Leyss., and *Poa compressa* L., on the other hand, appear to be immune under the same conditions.

Specimens of the field mustard (*Sinapis arvensis* L.) and wild oats (*Avena fatua* L.) found growing among the wheat plants in the unsterilized infested soil, were never found to harbor the parasite in question. To date, the fungus has only been found in soil from around Moose Jaw, Regina, Grenfell and Alameda, in the south of the Province.

Flask Experiments

Supplementary host-relationship studies were conducted on sterile seedlings of wheat, oats, barley and rye, in wide-mouthed 250 cc.. Erlenmeyer flasks containing about 5 cc. of sterile tap or distilled water. The seedlings were grown on filter paper in sterile moist chambers, and when the roots were one to three cm. long two or three seedlings were transferred under aseptic conditions to each flask and the cotton wool plug again inserted. At the same time several root tips heavily infested with sporangia of the wheat fungus in all stages of development, and in which there was no trace of any other parasite, were rinsed in sterile water and placed in the flasks. It had previously been ascertained that when such roots are placed in fresh sterile water, mature temporary sporangia discharge their contents into vesicles where the zoospores are formed and set free into the surrounding water. The zoospores on coming in contact with a root hair or epidermal cell could bring about infection.

On the second day after inoculation, thalli in all stages of development could invariably be observed within the root-tip cells of the wheat, barley, and rye seedlings, and not infrequently zoospore formation and discharge were

observed to take place. That is, a new generation of zoospores can be formed at least every 48 hours (17). The small size of the thallus allows of a very short period in the life-history of the individual. As each sporangium produces from a few to over 50 zoospores, the intensity of infection commonly found in infested roots of these cereals in potted soil is not at all surprising. Root tips from flask cultures inoculated two days previously were the best for observing zoospore penetration.

It was not found possible to continue observations on these roots after the third day as the cultures became too foul with bacteria which had gained entrance with the diseased roots, and the conditions of host and parasite were becoming morbid.

The leaves of the inoculated seedlings of wheat, barley, and rye turned a pale yellow which was usually apparent the second day after inoculation, whereas sterile seedlings in the uninoculated flasks used as controls remained relatively green.

In view of the fact that the organism has not yielded to the ordinary methods of laboratory culture, there is evidence that, under certain conditions at least, the organism can produce injurious effects on wheat, barley and rye seedlings.

Field Test Plots

In May, 1929, several sacks of soil taken from the top six inches were procured from the same field as that used in the pot tests. In the field tests, soil was removed to a depth of six inches from plots 2 ft × 3 ft, and six-inch boards were placed around the sides. These rectangular troughs were then filled with the infested soil. Several furrows three inches deep and one rod long were also filled with some of the infested soil.

Tests on varietal susceptibility of wheat, on host range on oats, barley, rye and flax, and on the effects of acid phosphate, of farmyard manure, and of packing, were conducted in these plots. No browning root-rot symptoms appeared on plants in any of the plots of infested soil; these yielded as well as the control plots of sterilized soil and of local soil. Twice a week seedlings with as much root system as possible were removed from each plot for examination. No lesioning of any consequence appeared on the roots, neither could any traces of the wheat fungus be found. Later, when the plants were about two months old, prolonged search would reveal an occasional lesioned root tip containing typical "browning bodies".

The dry weather which prevailed this season may have accounted for the non-appearance of browning root rot in the above test. The plots have been left undisturbed for a repetition of the experiment next season.

Owing to the exceptionally dry conditions which prevailed in the south of the Province where the soil used in the tests was obtained, it was impossible to separate drought injury from what may have been browning root rot. The crops in this locality were almost complete failures.

Preliminary Fertilizer Test

A preliminary experiment on the effects of different fertilizer treatments on the root rot was conducted in infested soil in earthenware crocks in the greenhouse. No outstanding differences of the effects of the various treatments on the incidence of the disease were obtained in this single series. Several repetitions of the experiment will be needed before definite conclusions can be drawn.

Further Biological Characters

Infested soil which has grown about six successive crops of wheat seedlings, *i.e.*, until they were three or four weeks old, still harbored the fungus after six months. The soil was kept in five-inch pots and was never allowed to become too dry. During the very hot greenhouse temperatures of the summer months the roots of wheat plants grown in these pots were practically free from the fungus, but when the pots were resown to wheat and placed outdoors in the shade of a stone building, the percentage of infected roots would again be high. It can be inferred from this that high temperature is not favorable to infection.

There was some indication too that at the higher temperatures more individuals conjugated in pairs to produce oospores than germinated by means of zoospores; this probably accounts for the scarcity of infection encountered above.

The formation of oospores could also be enhanced by allowing the soil to dry out slightly. Hence it would seem that adverse external conditions greatly determine sex in this organism. It would not be surprising, therefore, to find that, under the sudden environmental changes prevailing in nature, both temporary sporangia and oospores are formed concomitantly in the life-history of the organism.

No definite evidence has been obtained that mature temporary sporangia germinate by germ tubes at higher temperatures; therefore, with the exception of the tube of discharge, which occasionally may attain a length of over 20μ, and the vesicle, no extramatrical development of the organism has been observed.

The formation of vesicles could readily be initiated by adding fresh water to roots bearing mature temporary sporangia and keeping these at or just below room temperature. In one instance 20 to 30 vesicles were observed at the same time on one root tip in a low-power field of the microscope. This root had been removed from the soil and placed in a dish of water for about 25 minutes. It was then mounted in sterile water on a glass slide.

Cool temperatures and fresh moving water favor zoospore formation and discharge, and most probably infection of the wheat roots also.

Discussion of Systematic Position

As far as the authors have been able to ascertain, the fungus just described has not previously been reported. Its life-history and known characters do not even agree entirely with those of any one genus, but show it to have closest affinities with the genus *Lagenidium* of the family Ancylistaceæ of the class Phycomycetes.

It is at once excluded from the Olpidiaceæ in that it has more highly developed sexuality, in its method of zoospore discharge, and in its biflagellate zoospores.

With the Woroninaceæ (10), as exemplified by the genera *Olpidiopsis* and *Pseudolpidium*, it agrees in having bean-shaped zoospores with two lateral flagella. In both, the resting spore is produced by the fusion of two multinucleate cœnogametangia, but whereas in *Olpidiopsis* and *Pseudolpidium* the two conjugating thalli touch each other so that the contents of the smaller pass into the larger through a small opening at the point of contact, in the wheat fungus the contents of the male thallus pass *via* a definite antheridial tube into the female thallus, usually about the same size (13). In *Olpidiopsis*, the contents of more than one small male cell may be discharged into a single large female cell. In the wheat fungus there is some evidence to indicate that, at least, two male cells may transfer their contents to one female cell, although further observations on this point are needed. In *Olpidiopsis*, the male cell persists for a long time on the resting spore as an appendiculate cell, while in the wheat fungus the resting spore soon appears free from appendages of any kind. One of the outstanding differences between *Olpidiopsis* and *Pseudolpidium*, and the wheat fungus is that in the former genera the zoospore after entrance is free to move with the streaming protoplasm to different parts of the host cell; in the latter fungus, so far as is known, the zoospore always penetrates by a germ tube which enlarges into a germ-sphere and finally into an individual thallus, thus never being free within the living host cell. The Woroninaceæ are only parasitic on fungi and algæ. The foregoing facts indicate clearly that the wheat fungus does not belong to the Woroninaceæ.

It is in the characteristic formation of a gelatinous vesicle in the production and liberation of its zoospores that the wheat fungus shows very close affinities to the genus *Lagenidium* and the genus *Pythium*.

The entire contents of the zoosporangium, *i.e.*, a single thallus, of the wheat fungus are used up in the formation of zoospores. This apparently happens also in small, non-septate, poorly nourished, single individuals of *Lagenidium* which are morphologically very similar to the zoosporangium of the wheat fungus; but under favorable conditions of nutrition a mature, lobed, individual thallus of *Lagenidium* resulting from a single infection, may be cut off by septa into several potential zoosporangia or potential gametangia. Each zoosporangium of *Lagenidium* has its own exit tube and discharges its contents separately, the method of zoospore discharge in the two forms being identical.

The thallus of the wheat fungus, whether potentially a zoosporangium or a cœnogametangium, even under optimum conditions, always remains cœnocytic, and never shows pronounced lobulate branching. At most it is elongate-lobulate and occasionally hook-shaped, but when it is attached centrally to the cell wall of the host it tends to fill the whole cell, thereby frequently becoming somewhat bi-lobed (Fig. 5, *B*, *b;* and Plate I–5).

Comparison might also be made with *Pythium gracile* Schenk and the simpler species of *Pythium* of the subgenus *Aphragmium*. In *P. gracile*, mycelium is frequently very scanty, and almost its whole content is discharged as zoospores (9, pp. 74–75). There is no differentiated sporangium separated from the rest of the mycelium by a septum.

The zoospore of the wheat fungus also resembles that of *Lagenidium* and *Pythium*. It germinates by the passage of its contents into a germ-sphere within the host cell, as in *Lagenidium* and those species of *Pythium* parasitic in algæ (13, p. 97).

In the formation of its sexual spore the wheat fungus resembles the Ancylistaceæ (13, pp. 48 and 72). In both, the contents of the male thallus, the antheridium, pass along a conjugation tube into the female thallus, the oogonium, where the united plasmas contract and round off to form a thick-walled spore, the oospore. It could not be ascertained whether an oosphere forms in the oogonium before fertilization, as it does in the higher Oomycetes. But whereas the known members of the Ancylistaceæ may be either monœcious or dicœcious, the wheat fungus is apparently always dicœcious. The lack of formation of septa in mature individuals does not allow of monœcism with resulting formation of sexual spores in chains, and it is here that the wheat fungus differs fundamentally from the known genera of the Ancylistaceæ.

Physiologically, it differs from the Ancylistaceæ in being parasitic in the roots of Phanerogams. Although *Rhizomyxa hypogaea* Borzi, which is parasitic in the root hairs of different plants is placed in the Ancylistales by Shröter (9, pp. 89 and 91), it is now thought that it should be placed in the Plasmodiophorales (6, p. 304). Some doubt as to its identity has also been expressed.

In its parasitism, however, the wheat fungus again resembles many Pythiaceæ which show a decided preference for the root tips of flowering plants. To the other characters, already mentioned, which it has in common with the Pythiaceæ, may be added the great similarity between the mature oospore of the wheat fungus and those of certain species of *Pythium*. It differs from *Pythium* in its absence of mycelium and in its apparent strict parasitism.

It seems to the authors that the present study of the organism strengthens the view that the Ancylistaceæ and the Pythiaceæ are very closely related. The Ancylistaceæ may be considered intermediate between the Chytridiales on the one hand, and the Pythiaceæ on the other as shown by their respective types of sexual reproduction and the relative development of their thalli. Opinion seems to be divided as to whether the Ancylistaceæ should be regarded as an ascending line or as a degenerated one as a result of submerged parasitism. The same controversy will doubtless surround the systematic position of the wheat fungus (6, 10 and 13).

It has been shown that although the wheat fungus possesses several characters in common with certain genera of the Chytridiales, yet it cannot be placed in that order, although it may be considered as being at the upper end

of the Chytridiales series. Its absence of mycelium, its zoospore, sexual process, and type of sporangial germination, place it in the Ancylistaceæ, near the genus *Lagenidium*. In these respects too there is some suggestion that it might be considered as belonging to the lower end of the *Pythium* series, possibly a simplified *Pythium* possessing obligate parasitism.

On the basis of its sac-like cœnocytic thallus, its absence of definite branching, and its parasitism on the roots of Phanerogams, it is proposed to establish it in the new genus *Lagena* (from Latin *Lagena*, wine sac or large vessel with neck), with *radicicola* (from Latin *radix*, root; *colere*, to inhabit) as its specific designation.

Diagnosis

Lagena, n. gen.

Mycelium absent. Thallus sac-shaped or cylindrical, sometimes curved, unbranched, cœnocytic, with thin cell wall, attached to wall of host by a neck. May function as sporangium or cœnogametangium. Sporangium discharges its contents through an exit tube into a gelatinous vesicle in which the zoospores are formed. Zoospores bean-shaped, with two lateral flagella. Cœnogametangia in same cell conjugate in pairs, with an antheridial tube connecting the two gametes. Dicœcious. Oospore globose, smooth, and thick-walled with a large central globule and refringent spot. Obligate parasite.

Lagena radicicola, n. sp.

Mycelium lacking. Zoospore darkly granular, bean-shaped, with two flagella attached in the lateral depression; about 7μ by 11μ when motile and 6μ in diameter when at rest; rounds up on surface of host and penetrates it by a germ tube which enlarges and develops into a cœnocytic, sac-shaped, sometimes curved, occasionally elongate-globulate, multi-nucleate thallus. Thallus occupies cortical, epidermal and root hair cells of finer roots of host. Mature thalli occur singly or aggregated in one cell; they vary considerably in size, average 14 μ by 35 μ, are sac-shaped and attached to cell wall of host by a collar which in surface view appears as two concentric circles; may function as zoosporangia or cœnogametangia. Zoosporangium discharges its contents extramatrically through a single emission tube (4 μ wide by 10-15 μ long) into a vesicle where the zoospores, from a few to over 50, are formed and liberated. Contents of male thallus pass, *via* a conjugation tube, into female thallus, where a smooth, double-walled, sub-spherical resting spore (oospore) with central globule and refringent spot, is formed; size varies from 10 μ to 25 μ in diameter. Oospores also occur singly or several in one host cell. External conditions determine largely whether zoosporangia or oospores will be formed. Both zoosporangia and oospores may be formed concomitantly in the life-history of this organism. Germination of oospores has not been observed.

Obligate parasite; under experimental conditions parasitic on *Triticum æstivum* L., *T. durum* Desf., *Hordeum vulgare* L., *Secale cereale* L., and *Zea mays* L., causing discoloration and death of the fine root tips of seedlings grown in soil from near Regina, Saskatchewan.

Isolation of Pythium-like forms

During attempts to isolate *Lagena radicicola* in culture from root tips in which only temporary sporangia could be detected, occasional cultures of *Pythium* types were obtained.

Frequently, root tips, when kept in sterile water for a day or two, would develop a good growth of non-septate mycelium in the surrounding water. Then, by planting these root tips on water agar in a Petri plate, the mycelium would readily grow out over the agar, when hyphal-tip cultures could be procured. The usual difficulty of freeing isolations of Pythiaceous forms from bacteria was often encountered.

A systematic attempt was accordingly made to obtain fungous isolations from the roots of young wheat plants grown in pots in soil from browning root-rot fields from various localities. As soon as the seedlings began to show browning symptoms above ground, root tips, whether showing discoloration or not, were examined for the presence of internal mycelium, and isolations attempted according to the procedure outlined above. This method proved to be much more efficacious than the earlier attempt to obtain Pythium-like forms from the lesioned root tips packed with oospore-like bodies from field material. To date, more than a score of "isolation strains" of Pythiaceous forms have been secured by this method, and studies of their parasitism on cereals are now in progress. Several of these strains have been shown to be definitely parasitic on cereals in pot experiments both in the greenhouse and outdoors, producing symptoms both macroscopically and microscopically similar to those of browning root rot in the field. The oospores of *Lagena* and the parasitic *Pythium* strains are much alike, but whereas those of *Lagena* are confined to the epidermal and outermost cortical cells of the root, those of *Pythium* in greenhouse infected plants are found in the stele as well. As the oospores which are characteristic of browning root rot are found both in the stele and cortex, the above observation is evidence supporting the causal relation of *Pythium* species to the disease.

Conclusions

The early part of the present paper is an endeavor to present the browning root-rot situation and show that it is a definite problem needing urgent solution; the second part deals specifically with a fungus, apparently not hitherto described, which is found constantly associated with the fine root tips of wheat, barley, rye, and occasionally with maize plants, grown in soil from browning root-rot infested fields in the southern part of Saskatchewan. It is not an attempt to assign to this fungus, *Lagena radicicola*, the primary cause of the disease, but the authors are of the opinion that under certain conditions at least a heavy infection of root tips by the fungus will so reduce the rootlets that browning symptoms will appear on the plants above ground, growth will be retarded, and the yield slightly lessened. These conditions most probably prevail some seasons on the Regina Clay soil in the south of the Province, and here, at any rate, this fungus is likely one of the chief contributing causes of the disease. This soil contains parasitic *Pythium* forms as well.

However, when the Province as a whole is considered, the present studies, on the parasitism of *Pythium* forms isolated from the roots of cereals grown in infested soil, indicate that the disease under investigation is a *Pythium* root-rot problem of cereals, especially wheat, which is similar in many respects to the *Pythium* root rot complex of sugar cane in Hawaii (4), Louisiana (9), and elsewhere, and in some years equally as destructive and widespread as the *Helminthosporium-Fusarium* type of foot and root rot. Failure to obtain these parasitic *Pythium* forms from roots of field material collected late in the growing season, is thought to be due to the parasite's having entered the resting, oospore, stage; then, only organisms playing a secondary rôle could be isolated. This is supported by the fact that the parasitic "isolation strains" were readily obtained from the roots of wheat seedlings grown in pots of infested soil, in which case the isolations could be made when the parasite was still vegetatively active within the host tissues.

Investigations dealing specifically with browning root rot as related to *Pythium* strains are now in progress at this laboratory.

Acknowledgments

The authors wish to thank Professor W. P. Fraser, Department of Biology, University of Saskatchewan, for his interest in the work, for suggestions and criticisms during its progress, and for his help in securing necessary equipment; also Professor H. S. Jackson, Department of Botany, University of Toronto, for his critical reading of the section of the manuscript dealing with the new fungus.

References

1. Atkinson, G. F. Botan. Gaz. 48 (5):321-338. 1909.
2. Barrett, J. T. Ann. Botany 26:209-238. 1912.
3. Butler, E. J. Mem. Dept. Agr. in India, Bot. Ser. 1 (5):1-160. 1907.
4. Carpenter, C. W. Bull. Exp. Sta. Hawaiian Sugar Planters' Assoc. Bot. Ser. 3 (1):59-65. 1921.
5. Chabrolin, C. Abstract in Rev. Applied Mycol. 6:597-598. 1927.
6. Cook, W. R. I. New Phytologist 27 (4):230-320. 1928.
7. Drechsler, C. J. Agr. Research 38 (6):309-361. 1929.
8. Edgerton, C. W. Tims, E.C. and Mills, P. J. Phytopathology 19 (6):549-564. 1929.
9. Engler, A. und Prantl K. Die natürlichen Pflanzenfamilien. 1 Teil. 1 Abteilung. Leipzig. 1897.
10. Gaüman, E. A. Comparative Morphology of Fungi. (Translation by C. W. Dodge). McGraw Hill, New York, p. 701, 1928.
11. Gram, E. and Rostrup, Sofie. Abstract in Rev. Applied Mycol. 1:369-371. 1922.
12. Guyot, A. L. Ann. des Epiphyties 13 (2):79-93. 1927.
13. Gwynne-Vaughan, H. I. C. and Barnes, B. The Structure and Development of the Fungi. Cambridge Univ. Press, p. 384. 1927.
14. Joel, A. H., Hansen, R. and Wilson, A. M. Soil Survey Report No. 6, Univ. of Saskatchewan, Saskatoon, Canada. 1927.
15. Joel, A. H., Edmunds, F. H. and Mitchell, H. Soil Survey Report No. 7, Univ. of Saskatchewan, Saskatoon, Canada. 1928.
16. Johann, Helen et al. J. Agr. Research 37 (8):443-464. 1928.
17. Kusano, S. J. Coll. Agr. Imp. Univ. Tokyo. 4 (3):141-199. 1912.
18. Plant Disease Reporter, U.S. Dept. Agr., Suppl. 40:140. 1925.
19. Simmonds, P. M. Canada Dept. Agr. Div. Botany Report 1926:126-132. 1927.
20. Subramaniam, L. S. Agr. Research Inst. Pusa, Bull. 177:1-7. 1928.

CHEMICAL AND PHYSICO-CHEMICAL CHANGES IN WHEAT AND WHEAT PRODUCTS INDUCED BY ELEVATED TEMPERATURES

III. THE INFLUENCE OF GERM CONSTITUENTS ON BAKING QUALITY AND THEIR RELATION TO IMPROVEMENT IN FLOUR INDUCED BY HEAT AND CHEMICAL IMPROVERS[1]

By W. F. Geddes[2]

Abstract

Experiments were conducted in an effort to determine the cause of enhancement in baking quality previously observed on heat treatment of straight-grade flour not aged nor bleached. Heat treatment of straight-grade flour matured with agene (nitrogen trichloride), or of unaged fifth middlings flour (highly refined mill stream) did not result in any essential improvement in baking quality, and the unheated flours gave only a slight positive response to bromate. Similarly, ether extracted straight-grade flour gave no appreciable response to bromate and no significant improvement due to heat treatment. Germ added to fifth middlings flour markedly reduced its baking quality when determined by the basic procedure, as reflected in poorer handling qualities of the dough, and in the baked loaf by a decrease in loaf volume, underfermented characteristics, and coarse open texture. Increasing the fermentation time, addition of bromate, or heating the germ before admixture reduced the deleterious effects of the germ. The experiments indicate that response to bromate, and improvement of natural flour induced by proper heat treatment is associated with the presence of germ in the flour. Oxidation of certain germ constituents—presumably the phosphatides—is suggested as the primary change involved in such improvement. Addition of lecithin to middlings flour caused a marked response in loaf volume to the addition of bromate which is considered as indirect evidence that the phosphatides are involved. Heat treatment of germ induced a marked increase in the hydrogen ion concentration of aqueous extracts and a decrease in the iodine number of the ether extract. It is concluded from this series of investigations that heat treatment of flour is detrimental to gluten quality, but decreases the deleterious effect of germ present in the flour. Unaged flours containing low grade mill streams may show an enhancement in baking quality by heat treatment, but the improvement will not equal that induced by chemical improvers which apparently act primarily on the germ constituents.

Introduction

In a previous paper (3), the results of baking tests conducted on unbleached straight-grade flour milled from Western Canadian hard red spring wheat, which had been heat-treated at constant moisture content, were reported. Baking quality, determined by the standard method or basic procedure, (in which no chemical improvers are used) showed progressive improvement as the temperature or time of heating was extended within a certain range, beyond which marked damage to baking quality resulted. Baking tests by the bromate method (in which 0.001% potassium bromate was added to the baking formula) revealed in general, damage to baking quality with all heat treatments.

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Contribution from the laboratories of the University of Manitoba, Winnipeg, Manitoba, Canada, with financial assistance from the National Research Council of Canada. The present paper is the third of a series and is being issued as paper No. 8 of the Associate Committee on Grain Research. It represents part of a thesis presented in partial fulfilment of the requirements for the degree of Ph.D. at the University of Minnesota.

[2] *Professor of Agricultural Chemistry, University of Manitoba.*

Furthermore, the baking quality of the unheated straight-grade flour determined by the bromate method was superior to that of any of the heat treated flours when baked without bromate, which is in agreement with the conclusions of Berliner and Ruter (2) that improvement by heat is not comparable to that afforded by the use of chemical improvers. Moreover, the heat-treated flours showed a progressive decrease in the response to bromate as the severity of the treatment was increased, indicating that the action of heat is in the same direction as that of bromate and suggesting that the changes induced by heat may involve oxidation—the superimposing of an oxidizing agent on the already partially oxidized flour carrying the process too far.

In discussing the smaller improvement induced in baking quality by heating straight-grade flour than that produced by the use of potassium bromate in the baking formula, it was suggested, as a working hypothesis, that heat-treatment altered certain flour constituents in a direction associated with improved baking quality while others were changed in the reverse direction. It was assumed that the former effects were induced at a lower temperature or took place at a more rapid rate than the unfavorable changes—the resultant being an improvement in baking quality. The heat improvement, however, would be less than that brought about by the use of chemical improvers due to the unfavorable reactions produced at the same time.

The physico-chemical and chemical studies conducted on these flours failed to lend any firm support to the above hypothesis, although it explains the improvement in baking quality resulting from heat treatment. These studies, reported in a subsequent paper (4), showed, in general, that the changes induced by heat were in the direction of decreased strength. Gluten quality was impaired in all heat-treated samples, as evidenced by a decreased viscosity of leached acidulated flour suspensions, a decreased rate and extent of imbibition of the washed out gluten, and decreased gas retention of the dough. Heat treatment resulted in decreased protein peptization by N. magnesium sulphate and N. potassium iodide solutions which, however, was closely correlated with decreased viscosity and decreased gas retention, and hence did not indicate increased strength in this series. Diastatic activity, although showing no marked change in the region of improvement, showed slight decreases as determined by the modified Rumsey method as well as by the rate of gas production in doughs. Proteolytic activity of the flours was markedly decreased with heat treatment, significant decreases being noted before diastatic activity was appreciably altered. In view of the relatively high proteolytic activity of yeast, and the lower gas retaining power of the doughs made from these flours, the decreased proteolytic activity resulting on heat treatment does not explain the improvement in baking quality.

No chemical work, however, was undertaken on the lipoid and ash constituents of these flours. As a further test of the working hypothesis set up, several experiments were conducted which, while far from complete, led to such astonishing results that it seemed advisable to present a preliminary report at this time.

Experimental

Influence of Heat Treatment on Agene-treated Straight-grade Flour

The investigations on the influence of heat treatment on baking quality previously reported (3), were limited to untreated straight-grade flour of 74% extraction, and the question naturally arose whether bleached flour would show improvement in baking quality on similar heat treatment.

Portions of the original straight-grade flour were bleached with agene (nitrogen trichloride) in the experimental bleacher supplied by Wallace and Tiernan Company, the dosage being at the rate of three grams per barrel. The flour was thoroughly mixed and bolted after bleaching and heat treatments conducted for varying times at 155° F. (the moisture content of the flour was 13.46% determined by the vacuum oven method), in the apparatus previously described (3). This temperature was chosen, since in previous experiments, it was found to give both improvement and damage to baking quality (basic procedure) by varying the time of heating. The flours, including the original unbleached flour, were baked with and without the addition of 0.001% potassium bromate, the mean results of duplicate bakings being recorded in Table I.

For comparison, the baking quality of the original unbleached flour similarly heat treated is reproduced from a previous paper (3); in which details of the baking procedure and method of scoring the baked loaf are given.

The agene-treated flour, in contrast with the unbleached sample, showed no essential improvement when baked by the basic procedure for the short periods of heating, and a somewhat more marked degradation in baking quality as the time of heating was extended. The response of the bleached flour to bromate was, as has been noted by other workers, much less than that of the untreated flour. The superimposing of heat treatment on the bleached flour resulted in a very marked degradation to baking quality as determined by bromate. The damage was reflected in decreased loaf volume, over fermented crust characteristics and coarse texture of the crumb. The results indicate that the action of heat on the one hand, and bromate and agene on the other, is in part at least, in the same direction, and suggest that the improving effect of heat may possibly be ascribed to a similar action as that of chemical improvers on some constituents of the flour.

Influence of Heat on Ether Extracted Straight-Grade Flour

A few preliminary heat treatments conducted on an untreated fifth middlings flour, the highest grade mill stream obtainable from a local flour mill, revealed no improvement in baking quality, and the unheated flour gave only a very slight positive response to bromate. These observations suggested that the improvement in baking quality induced by heat and chemical improvers might be due to some action on the germ constituents which are undoubtedly present in straight-grade flour. If this were the case then partial removal of these constituents from straight-grade flour should reduce the response to heat and chemical improvers.

TABLE I

INFLUENCE OF TIME OF HEATING AT 155° F. ON THE BAKING QUALITY OF UNTREATED AND AGENE-TREATED STRAIGHT-GRADE FLOUR. (MOISTURE CONTENT OF UNTREATED FLOUR, 13.90%, OF AGENE-TREATED FLOUR, 13.46%)

Heat Treatment		Nature of flour	Absorption	Loaf volume, in cc.	General appearance	Crust color	Grain and texture	Single figure estimate
Temperature, in ° F.	Time, in hr.							
Basic Procedure								
Control		Unbleached	61	552	3.4 g	5.0	5.7 C	81
Control		Bleached	65	593	4.2	5.0	7.4 C	91
155	0.5	Unbleached	62	575	3.2 g	5.0	7.2 C	87
155	0.5	Bleached	65	584	4.7	4.8 P	8.4	93
155	1.0	Unbleached	62	565	3.3	5.0	8.2	89
155	1.0	Bleached	66	561	4.6	4.8 P	8.3	90
155	2.0	Unbleached	62	541	3.3	5.0	8.1	87
155	2.0	Bleached	66	554	3.9	4.2 P	8.3	88
155	3.0	Unbleached	62	555	3.4	5.0	8.3	89
155	3.0	Bleached	66	521	3.4 O	4.4 P	8.0	84
155	4.0	Unbleached	62	527	3.3 O	4.3 P	8.1	85
155	4.0	Bleached	65	468	2.5 O	3.6 P	5.6 C&c	70
155	6.0	Unbleached	61	496	3.4 O	4.3 P	7.0 C	78
155	6.0	Bleached	64	475	2.4 O	3.6 P	7.5	76
155	8.0	Unbleached	60	500	3.7 O	4.2 P	7.5	80
155	8.0	Bleached	64	424	2.6 O	3.3 P	6.4 C&c	68
155	10.0	Unbleached	61	441	2.7 O	3.3 P	6.4 C&c	69
155	10.0	Bleached	64	435	2.1 O	3.3 P	7.3	71
Basic Procedure +0.001% Potassium Bromate								
Control		Unbleached	61	636	5.0	5.0	8.2	98
Control		Bleached	65	660	5.0	5.0	8.8	102
155	0.5	Unbleached	62	611	4.4	4.9 P	8.8	97
155	0.5	Bleached	65	580	4.2	4.6 P	8.8	93
155	1.0	Unbleached	62	593	4.3	5.0	8.3	94
155	1.0	Bleached	66	534	3.9	4.4 P	7.4	84
155	2.0	Unbleached	62	558	3.9	4.8 P	7.6	87
155	2.0	Bleached	66	497	3.9	4.2 P	7.5	80
155	3.0	Unbleached	62	522	3.1 O	4.5 P	7.0	81
155	3.0	Bleached	66	487	3.3 O	4.3 P	7.3 C	78
155	4.0	Unbleached	62	487	3.4 O	4.5 P	6.6 C&c	76
155	4.0	Bleached	65	449	2.9 O	3.7 P	6.7 C&c	72
155	6.0	Unbleached	61	425	2.4 O	3.3 P	5.0 C&c	63
155	6.0	Bleached	64	389	2.6 O	2.8 P	4.9 C&c	59
155	8.0	Unbleached	60	452	2.6 O	4.0 P	6.5 C&c	71
155	8.0	Bleached	64	366	2.0 O	2.1 P	4.7 C&c	55
155	10.0	Unbleached	61	352	1.9 O	2.9 P	4.5 C&c	54
155	10.0	Bleached	64	350	2.0 O	2.1 P	4.6 C&c	53

Johnson (5) has shown that ether extraction of flours results in marked improvement in color, texture, and in most cases loaf volume, yet no significant differences between the natural and extracted flours in regard to quality and quantity of gluten, absorption, and viscosity of leached acidulated suspensions, were observed. Diastatic activity, however, was considerably increased by

extraction. The improvement in baking quality due to extraction was more marked in the lower grade of flour. From his observations on the effect of adding lard to the ether extracted flour, it was concluded that constituents other than true glycerides of the fatty acids were responsible for the poorer baking quality of the natural flour.

In the present study, 2,500 gm. of the original straight-grade flour was extracted successively with four five-pound lots of ethyl ether, the ether being removed by filtration on a Büchner funnel. After the last extraction, the ether remaining in the flour was allowed to evaporate at room temperature and the flour placed in a humidified cabinet, in order to secure a moisture content closely approximating that of the original flour. The extracted flour, as noted by Winton (7) and Johnson (5), resembled powdered starch in its physical properties. Heat treatments were run on portions of this flour (13.96% moisture) at 140° F. for six hours and 143° F. for three hours (temperatures and times

TABLE II

SHOWING THE INFLUENCE OF ETHER EXTRACTION ON BAKING QUALITY OF STRAIGHT GRADE FLOUR AND CHANGES IN BAKING QUALITY PRODUCED BY HEAT

Sample	Treatment		Loaf volume, in cc.	General appearance	Crust color	Grain and texture	Single figure estimate
	Temperature, in ° F.	Time in hr.					
Basic Procedure							
Original St. Grade	Control		552	3.4 g	5.0	5.7 C	81
Original St. Grade	140	6	567	3.2	5.0	8.0 C	89
Original St. Grade	145	3	553	3.3	5.0	8.3 C	89
Ether Extracted	Control		603	4.6 g	5.0	9.3	98
Ether Extracted	140	6	608	4.8 g	5.0	9.9	100
Ether Extracted	145	3	607	4.8 g	5.0	9.8	100
Basic Procedure+0.001% pot. bromate							
Original St. Grade	Control		636	5.0	5.0	8.2	98
Original St. Grade	140	6	582	4.1	4.3 P	8.0	91
Original St. Grade	145	3	601	4.3	4.9 P	8.1	94
Ether Extracted	Control		647	4.2 O	4.8 D	9.6	103
Ether Extracted	140	6	585	4.1 O	4.9 D	9.9	97
Ether Extracted	145	3	584	3.9 O	4.9 D	9.8	97

which showed marked improvement in baking quality with the unextracted flour). The samples were baked with and without bromate in comparison with the unheated ether extracted and original flour. The results are recorded in Table II. For the purpose of comparison, data for the corresponding heat treatments on the original unextracted flour are also given. Photographs of the loaves are presented in Plate I.

Marked improvement in baking quality (without bromate) of the control flour resulted from ether extraction. The "green" or underfermented appearance was greatly reduced, and the loaves had a "bolder" appearance with

good break and shred. Crumb color (not recorded in the Table) and texture were markedly improved. When baked with bromate, the improvement resulting from ether extraction alone was not so pronounced. The ether extracted sample gave loaves showing signs of over fermentation. Ether extraction markedly reduced the response to bromate, suggesting that the ether has induced in the flour changes similar to those of bromate or that the flour constituents on which potassium bromate chiefly acts have been largely removed by the ether.

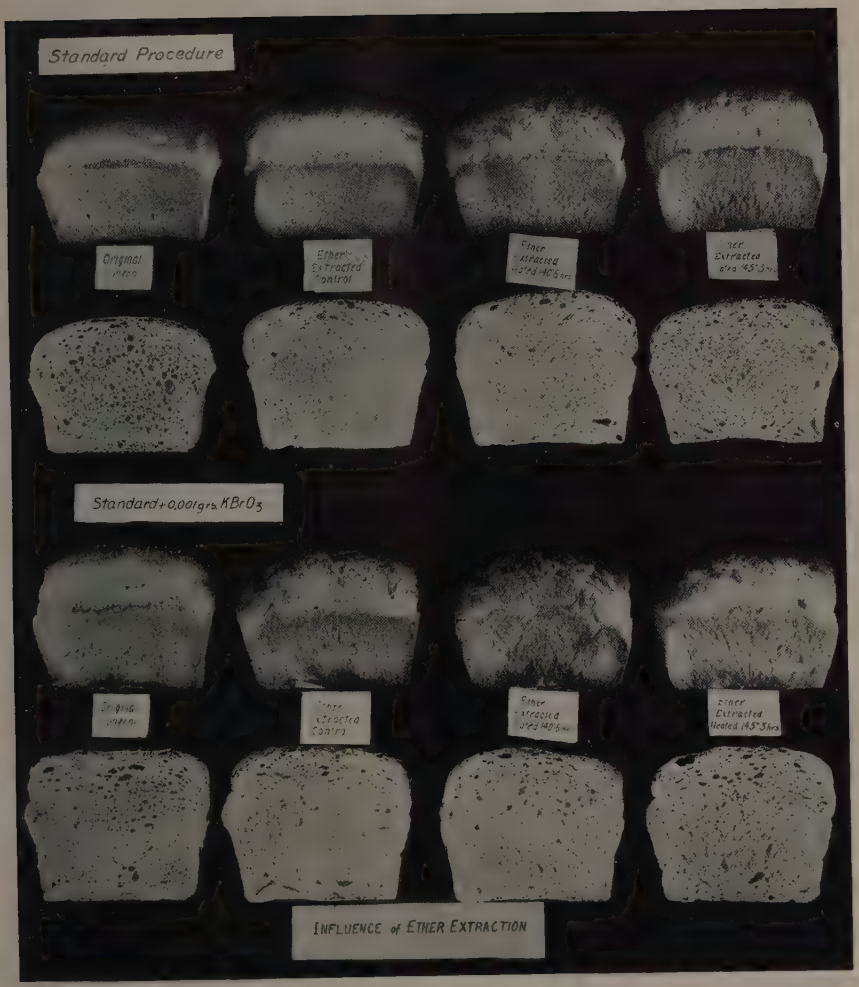
Furthermore, heat treatment of ether extracted flour gave no significant improvement in baking quality. These results are considered as evidence that alterations in certain ether soluble constituents of flour are in large measure responsible for the improvement in baking quality observed on heat treatment and also on treatment with chemical improvers.

TABLE III

BAKING RESULTS—BASIC PROCEDURE
INFLUENCE OF TIME OF HEATING AT 155° F. ON BAKING QUALITY OF FIFTH MIDDLINGS FLOUR AND ON THE DELETERIOUS EFFECT OF 5% ADDED GERM (HEAT TREATED VS UNTREATED)

Treatment*		Loaf volume in cc.	General appearance	Crust color	Grain and texture	Single figure estimate
Temperature, in ° F.	Time, in hr.					
M Control		608	4.1 g	4.8 P	8.3 C&O	95
M+raw germ		562	2.3 g	4.6 D	2.7 C&O	71
M155–0.5		565	3.7 g	5.0	8.1 C	89
M155–0.5+raw germ		549	2.7 g	4.5 D	3.1 C&O	71
M+germ 155–0.5		568	2.2 g	4.6 D	2.7 C&O	72
M155–1		553	3.9 g	4.9 D	8.5	90
M155–1+raw germ		529	2.5 g	5.0	3.0 C&O	69
M+germ 155–1		580	2.3 g	4.6 D	2.8 C&O	73
M155–2		540	4.5 g	5.0	8.4 C&O	89
M155–2+raw germ		552	3.4 g	4.6 D	3.9 C&O	75
M+germ 155–2		594	2.3 g	4.8 D	2.7 C&O	75
M155–3		520	4.4	4.8 P	8.3 C&O	86
M155–3+raw germ		525	3.2	4.6 D	3.8 C&O	72
M+germ 155–3		595	2.6 g	4.5 D	3.5 C&O	77
M155–4		505	3.7 O	4.8 P	8.0 C	83
M155–4+raw germ		531	3.0 O	4.8 D	5.1 C&O	76
M+germ 155–4		593	2.6 g	4.3 D	4.0 C&c	78
M155–6		488	3.7 O	4.6 P	7.4 C&c	79
M155–6+raw germ		525	3.4 O	4.5 D	6.5 C&O	80
M+germ 155–6		577	2.8 g	4.6 D	3.0 C&O	74
M155–8		460	2.6 O	4.0 P	7.3 C&c	75
M155–8+raw germ		500	3.3 O	4.8 P	5.7 C&c	75
M+germ 155–8		555	2.4 g	4.5 D	4.8 C&c	77
M155–10		413	2.1 O	3.3 P	6.3 C&c	66
M155–10+raw germ		476	2.6 O	4.4 P	5.7 C&c	72
M+germ 155–10		599	2.6 g	4.5 D	4.1 C&O	79

The treatment is designated by recording the temperature in ° F. followed by the time of heating in hours, e.g., "M155–0.5+raw germ" designates fifth middlings flour heat treated at 155° F. for 0.5 hr. to which was added 5% unheated germ.



The influence of ether extraction on the baking quality of straight-grade flour, and on the change in baking quality induced by heat treatments.

The Effect of Heat Treatment on Germ

Mr. A. W. Alcock, (Chief Chemist, Western Canada Flour Mills, Winnipeg) in a personal communication, called the writer's attention to some unpublished experiments he conducted in 1924, in which wheat germ to the extent of 3% was added to a short patent flour, resulting in a marked decrease in baking quality of the latter. Acidulation of the dough did not result in any reduction of the harmful effects of the germ, which indicated that its deleterious influence was not due to buffer salts. In this communication, he reported also that Arkady in the baking formula greatly improved the baking quality of the germ-patent mixture. Natural aging or heating the germ to temperatures above 100° C. was observed to decrease also the harmful effects on the baking quality of patent flour.

This information suggested a series of experiments to determine separately, in so far as was possible, the influence of heat on the endosperm and the germ constituents. A quantity of untreated fifth middlings flour (as representing the endosperm) and also a sample from the purest germ stream was obtained.

TABLE IV

BAKING RESULTS BASIC PROCEDURE + 0.001% POTASSIUM BROMATE
INFLUENCE OF TIME OF HEATING AT 155° F. ON BAKING QUALITY OF FIFTH MIDDLINGS FLOUR AND ON THE DELETERIOUS EFFECT OF 5% ADDED GERM (HEAT TREATED VS UNTREATED)

Treatment*	Loaf volume in cc.	General appearance	Crust color	Grain and texture	Single figure estimate
M Control	627	5.0	5.0	9.3	101
M+raw germ	632	3.2 g	4.5 D	6.5 C&O	90
M155–0.5	553	4.2 O	4.8 P	9.1	92
M155–0.5+raw germ	613	3.7 g	4.5 D	6.8 C&O	90
M+germ 155–0.5	639	2.9 g	4.5 D	6.5 C&O	91
M155–1	516	4.2 O	4.6 P	9.0	87
M155–1+raw germ	599	3.8 g	4.8 D	7.3 C&O	90
M+germ 155–1	647	3.9 g	4.5 D	6.6 C&O	93
M155–2	511	4.1 O	4.8 P	8.9	87
M155–2+raw germ	573	3.7 O	4.8 D	7.4 C&O	88
M+germ 155–2	642	3.0	4.8 D	6.0 C&O	90
M155–3	430	2.3 O	4.4 P	7.5 C&c	72
M155–3+raw germ	553	3.3 O	4.8 D	6.9 C	84
M+germ 155–3	643	3.9 g	4.5 D	6.7 C&O	93
M155–4	422	2.0 O	3.9 P	6.7 C&c	68
M155–4+raw germ	556	3.2 O	4.9 D	7.0 C&O	85
M+germ 155–4	650	4.3	4.4	7.0 C&O	95
M155–6	387	1.4 O	3.0 P	6.0 C&c	61
M155–6+raw germ	498	2.6 O	4.9 P	6.4 C&c	76
M+germ 155–6	663	4.3	4.5 D	7.3 C&O	97
M155–8	320	1.1 O	2.0 P	5.0 C&c	50
M155–8+raw germ	474	2.6 O	4.7 P	6.1 C&c	73
M+germ 155–8	667	4.3	4.3 D	7.7 C&O	98
M155–10	301	1.1 O	1.8 P.	4.8 C&c	47
M155–10+raw germ	420	1.9 O	3.5 P	5.4 C&c	64
M+germ 155–10	650	4.2 O	4.4 D	7.3 C&O	96

**The treatment is designated by recording the temperature in ° F. followed by the time of heating in hours, e.g., "M155–0.5+raw germ" designates fifth middlings flour heat treated at 155° F. for 0.5 hr. to which was added 5% unheated germ.*

(The germ was contaminated to some extent with bran chips, and a little flour). The germ was ground in a Wiley laboratory mill, the 0.5 mm. screen being used. As the bran pulverized less readily, it was largely removed by this means.

Heat treatments for varying times were conducted separately on the fifth middlings flour and on the germ at 155° F. (moisture content of the flour by the vacuum oven method being 14.20%, and that of the germ 11.56%).

A series of baking tests, with and without bromate, were run on the heated middlings alone, unheated middlings +5% heated germ, and heated middlings +5% unheated germ. Samples of unheated middlings alone and unheated middlings + 5% "raw" (unheated) germ were baked as controls. The detailed baking data with and without bromate are recorded in Tables III and IV. The summarized results are given in Table V.

TABLE V

SUMMARY OF BAKING RESULTS

INFLUENCE OF TIME OF HEATING AT 155° F. ON BAKING QUALITY OF FIFTH MIDDLINGS FLOUR AND ON DELETERIOUS EFFECT OF 5% ADDED GERM (HEAT TREATED VS UNTREATED)

Treatment*	Loaf volume			Single figure estimate		
	Basic procedure cc.	Basic procedure +0.001% $KBrO_3$ cc.	Reaction to bromate cc.	Basic procedure	Basic procedure +0.001% $KBrO_3$	Reaction to bromate
M Control	608	627	+19	95	101	+ 6
M+raw germ	562	632	+70	71	90	+19
M155–0.5	565	553	−12	89	92	+ 3
M155–0.5+raw germ	549	613	+64	71	90	+19
M+germ 155–0.5	568	639	+71	72	91	+19
M155–1	553	516	−37	90	87	− 3
M155–1+raw germ	529	599	+70	69	90	+21
M+germ 155–1	580	647	+67	73	93	+20
M155–2	540	511	−29	89	87	− 2
M115–2+raw germ	552	573	+21	75	88	+13
M+germ 155–2	594	642	+48	75	90	+15
M155–3	520	430	−90	86	72	−14
M155–3+raw germ	525	553	+28	72	84	+12
M+germ 155–3	595	643	+48	77	93	+16
M155–4	505	422	−83	83	68	−15
M155–4+raw germ	531	556	+25	76	85	+ 9
M+germ 155–4	593	650	+57	78	95	+17
M155–6	488	387	−101	79	61	−18
M155–6+raw germ	525	498	−27	80	76	− 4
M+germ 155–6	577	663	+86	74	97	+23
M155–8	460	320	−140	75	50	−25
M155–8+raw germ	500	474	−26	75	73	− 2
M+germ 155–8	555	667	+112	77	98	+21
M155–10	413	301	−112	66	47	−19
M155–10+raw germ	476	420	−56	72	64	− 8
M+germ 155–10	599	650	+51	79	96	+17

The treatment is designated by recording the temperature in ° F. followed by the time of heating in hours, e.g., "M155-0.5 - raw germ" designates fifth middlings flour heat treated at 155° F. for 0.5 hr. to which was added 5% unheated germ.

This series of experiments gave surprising results, but are somewhat difficult to interpret owing to the complexity of the flour and germ mixtures.

The addition of 5% "raw" germ to unheated middlings flour resulted in a very sticky dough of inferior handling qualities, which, however, improved considerably as the fermentation proceeded. When baked without bromate, the loaves exhibited pronounced characteristics of a green or under-fermented dough, namely, flat top, glossy sides, and no break. Loaf volume was considerably reduced, compared with the volume of fifth middlings baked alone, and the texture was very coarse and open. When baked with bromate, the middlings flour showed a slight improvement in baking quality, but the middlings+5% "raw" germ gave a very marked response in regard to handling qualities, loaf volume general appearance and texture—the response in loaf volume being 70 cc. and in single figure estimate 19. These changes are evident in the photographs in Plate II. The addition of bromate largely removed the deleterious effects of the germ on the baking quality of the middlings flour.

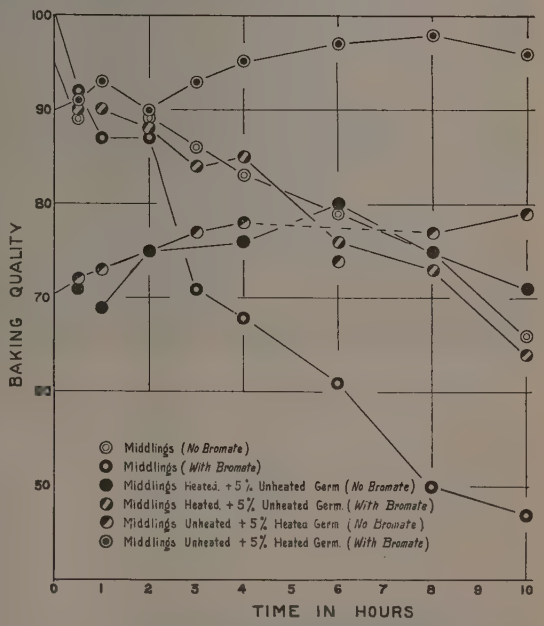


FIG. 1. *Influence of increasing time of heating at 155° F. on baking quality of fifth middlings flour (14.20% moisture content) and on the deleterious effect of 5% added germ.*

The influence of heat treatment on the germ can be most readily observed from the graphical presentation in Fig. 1.

Heat treatment of the middlings alone resulted in *no improvement* but a progressive decrease in baking quality as determined both with and without bromate, the response to bromate becoming more strongly negative as the time of heating was extended. These results indicate that the action of heat and potassium bromate exerts a harmful influence on the endosperm constituents.

Addition of 5% "raw" germ to the heated middlings flour yielded surprising results when baked without bromate. Increased time of heating the middlings flour up to six hours with the subsequent addition of "raw" germ showed continuous improvement in baking quality. In other words an increased heat treatment of the middlings flour reduced the deleterious effect of the addition of "raw germ", with the result that the baking quality (without bromate) of

the heated "middlings+'raw' germ" mixture was equal or slightly superior to that of the heated middlings alone, when the time of heating exceeded six hours. The addition of raw germ to the heated middlings delayed the onset of the overaged appearance of the loaves and improved the handling qualities of the doughs. (The over-heated middlings yielded doughs which lacked resiliency and the characteristic "dead" feeling of these doughs was considerably reduced by the addition of "raw" germ). The results indicate that the unheated or "raw" germ increased the fermentation tolerance of the over-heated middlings flour.

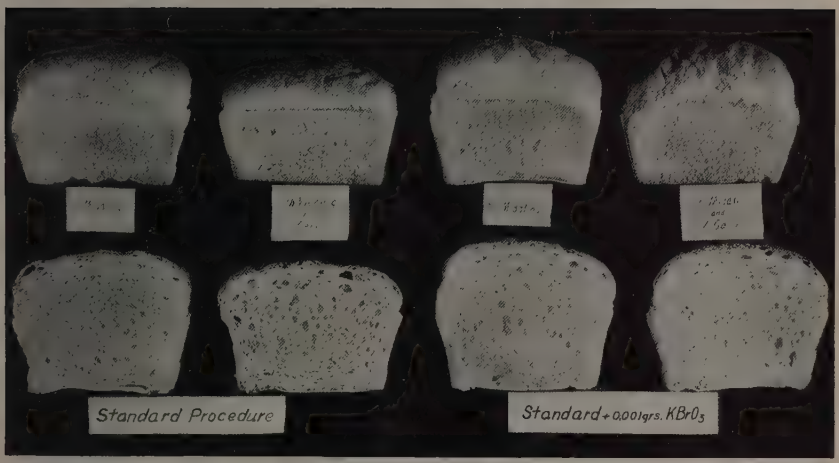
When baked with bromate, the baking quality of the heated middlings+"raw" germ mixture decreased with increasing time of heating, but was again superior to that of the heated middlings baked alone (with the exception of the short treatments).

The baking results of the heated "germ+unheated middlings" series are of considerable interest, a progressive improvement in baking quality (with and without bromate) being obtained with increased time of heating the germ. In other words, heating the germ lessens its deleterious effect on the baking quality of the middlings flour. Heating the germ progressively reduced its effect on producing the "green" crust characteristics, and the top of the loaf took on a more rounded shape. The texture was also markedly improved as time of heating the germ was extended. The photographs in Plate III illustrate quite well the noticeable decrease in the harmful influence of the germ constituents brought about by heat treatment.

These experiments seem to offer an explanation of the improvement in baking quality observed in the heat treatment of the straight-grade flour. The influence of heat on the endosperm constituents is, in general, entirely in the direction of decreased baking quality, while, on the other hand, heat treatment alters the germ constituents in a direction which reduces their harmful effects. When a straight-grade flour is heated both effects are taking place concomitantly. At the lower heat treatments it is assumed that the beneficial effects on the germ more than offset the detrimental influence of heat on the endosperm constituents, so that some improvement in baking quality results. It is true that in the experiments reported above, the germ was not altered to any appreciable extent in the shorter times, i.e., those which showed marked improvement in the baking quality of straight-grade flour. It is suggested that the lower moisture content (11.56%) at which the germ was heated resulted in a lengthening of the time required to cause a marked reduction in its deleterious effects.

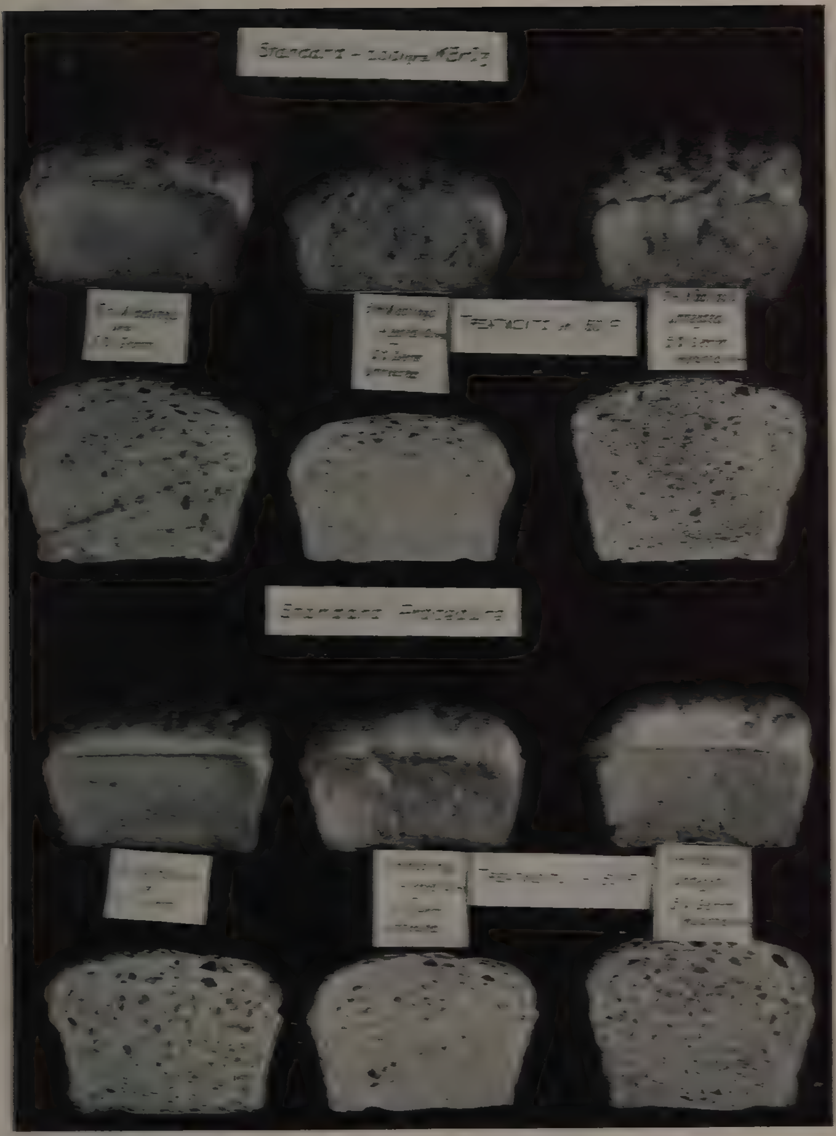
This theory is supported by the results of the physico-chemical and chemical studies conducted, and explains the markedly decreased and almost entire removal of any improvement, by means of heat, of ether extracted straight-grade flour.

In this connection, the statement of Kent-Jones (6) is interesting, "A method of systematically heating the flour from the bottom of the mill gives results which are truly astonishing."



The effect of 5% additions of "raw" germ on the baking quality of fifth middlings flour as determined with and without bromate.

Plate III



[illegible]

Furthermore, these experiments suggest that the beneficial effect of potassium bromate is due to a similar cause. The action of bromate appears to be primarily on certain germ constituents. It is well known that the low grade streams show greater responses to bromate than the more highly refined flours. These streams are higher in protein content and their more marked response has been attributed to this fact. The results obtained here are very strong evidence that the bromate exerts its beneficial action directly on some germ constituent, since the addition of 5% germ to patent flour did not materially alter its protein content. It is hardly conceivable that the addition of germ to middlings flour altered the inherent qualities of the gluten proteins. It seems probable that the oxidation of some germ constituent allows the inherent properties of the gluten to come into play, and it would be expected that the higher the protein content, the greater would be the response to bromate. In the physico-chemical studies, previously reported, (4), it was found that loaf volume, determined with bromate added to the basic formula, was more closely correlated to gluten quality than with the basic procedure. If the writer's premises are valid, the relation between bromate and gluten quality is largely an indirect one.

The question naturally arises as to what physico-chemical or chemical changes are induced by heating germ or by the use of potassium bromate. From the results of the ether extraction of straight-grade flour, it seems probable that the changes involved are to be found in the ether extractible constituents. The observations of Working (8, 9, 10) are of interest in this connection. He has pointed out the importance of the phosphatides, suggesting that they exert a lubricating effect on the gluten strands of the dough, allowing them to slip more readily upon each other. It is assumed that the phosphatide is so constructed that it cannot spread over the interfaces between the gluten fibrils. He states: "It seems probable that the action of oxidizing agents consists in breaking up these combinations so that phosphatides can be dispersed in the water present."

It seemed of interest to ascertain whether heating lecithin would in any way alter its effect on baking quality, as observed by Working (8). Ten grams egg yolk lecithin (labelled 90% Merck & Co.) was spread out on a glass plate and heated for ten hours at 100° C., no provision being made for maintaining the presence of moisture. Baking tests (with and without bromate) were then conducted on fifth middlings flour to which 1.0% of the heated lecithin had been added, in comparison with a similar addition of the original lecithin. The results are recorded in Table VI, and photographs of representative loaves shown in Plate IV.

TABLE VI

BAKING RESULTS

INFLUENCE OF LECITHIN

Treatment	Loaf volume, in cc.	General appearance	Crust color	Grain and texture	Single figure estimate
Basic Procedure					
M* Control	608	4.1 g	4.8 P	8.3 C&O	95
M+1.0% Lecithin	679	4.2 g	4.8 P	7.5 C	99
M+1.0% Heated lecithin	685	4.1 g	4.6 P	7.1 C	98
Basic Procedure + 0.001% pot. bromate					
M Control	627	5.0	5.0	9.3	101
M+1.0% Lecithin	748	3.4 O	4.9 P	8.3	108
M+1.0% Heated lecithin	755	3.4 O	4.4 P	8.4	109

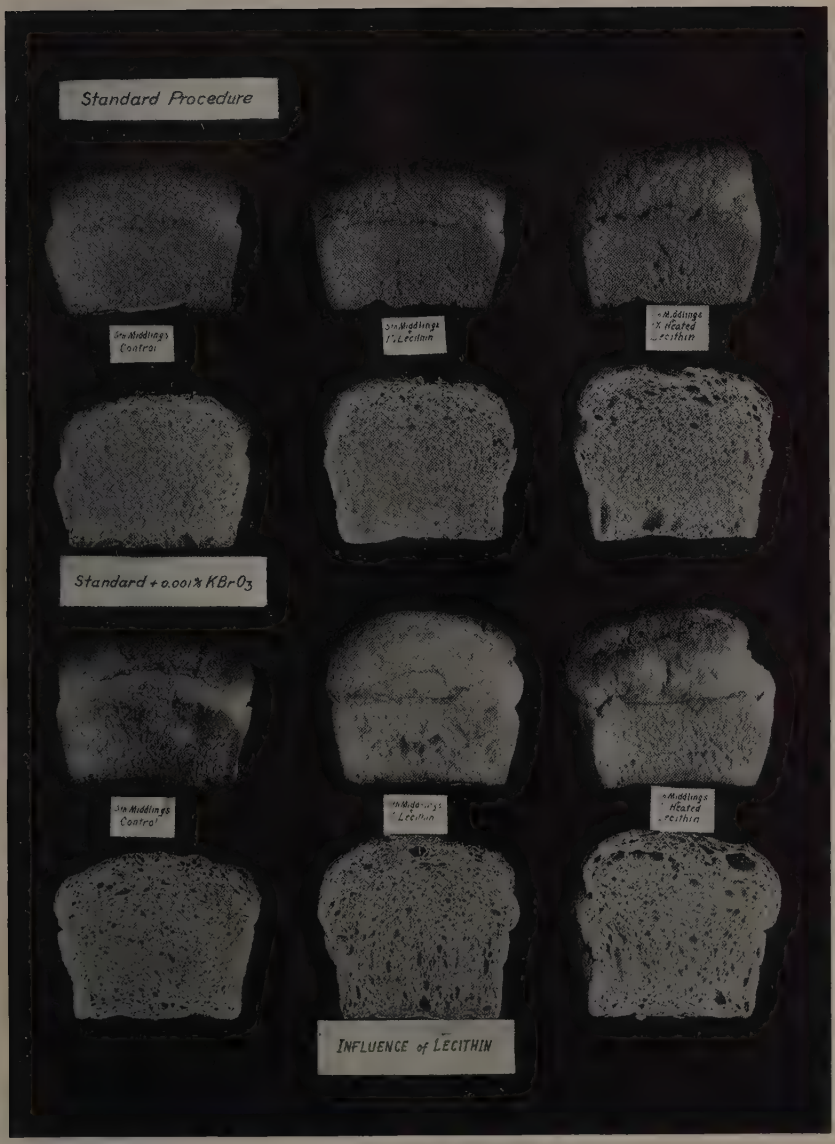
**M = fifth middlings flour.*

The results of this preliminary experiment are not conclusive with regard to the effect of heating, but they are interesting in that they show that the addition of lecithin to middlings flour causes a very marked response to bromate, which is accompanied by an "aged" appearance of the loaf. This is considered as indirect evidence that the action of oxidizing agents is on the phosphatides of the wheat germ, as has been previously suggested by Working (9, 10).

Influence of Heating Germ at varying temperatures on the Baking Quality of Flour-germ Mixtures.

The heating of germ for increasing times at 155° F. resulted in a continuous decrease in its harmful effects on the baking quality of fifth middlings flour, as determined without bromate. With bromate added, however, there was a tendency towards a falling off in baking quality for the 10-hour germ treatments, which suggested that the reactions, presumably oxidation, could be carried too far, with a resulting decrease in fermentation tolerance.

To secure information on this point, wheat germ was heated at various temperatures, increasing by intervals of 20° F., up to 250° F., for three hours (moisture content of germ 11.56%). No appreciable change in the physical properties of the germ was noted until the temperature of 210° F. was reached, when the color was observed to become slightly darker. At the higher temperatures, however, very marked changes were observed. The color became much darker, and the smooth "silky feel" of the ground germ was replaced by a harsh feeling and the material ran through the fingers much like fine sand. The odor somewhat resembled that of cocoa. These changes were most evident in the case of the treatment at 250° F.



The influence of the addition of 1.0% lecithin (heated vs unheated) on the baking quality of fifth middlings flour as determined with and without bromate.

Mixtures of 5% heated germ and 95% untreated fifth middlings flour were made and baking tests conducted. The samples were baked, without bromate, the fermentation time being three hours, and also with bromate, using a fermentation time of 1.75, 3.0 and 4.25 hours, in order to secure a measure of fermentation tolerance.

The detailed baking results are recorded in Table VII, and a summary given in Table VIII.

Interesting differences in the handling quality of the doughs were noted, and these were evident even on removing the doughs from the mixer. As was previously noted, the addition of "raw" germ resulted in a very sticky dough which improved in handling qualities as the fermentation proceeded. The improvement was very marked when bromate was used, particularly early in the fermentation.

The deleterious effect of the germ on handling qualities was markedly reduced by increasing the heat treatment of the germ. Without bromate, progressive improvement was noted as the fermentation time was extended. With bromate the higher heat treatments exhibited an improvement to an optimum condition, at which time the doughs behaved on handling very much like those made from a highly refined flour. As the time of fermentation increased beyond this point, the doughs had an excessively tough and dry feeling.

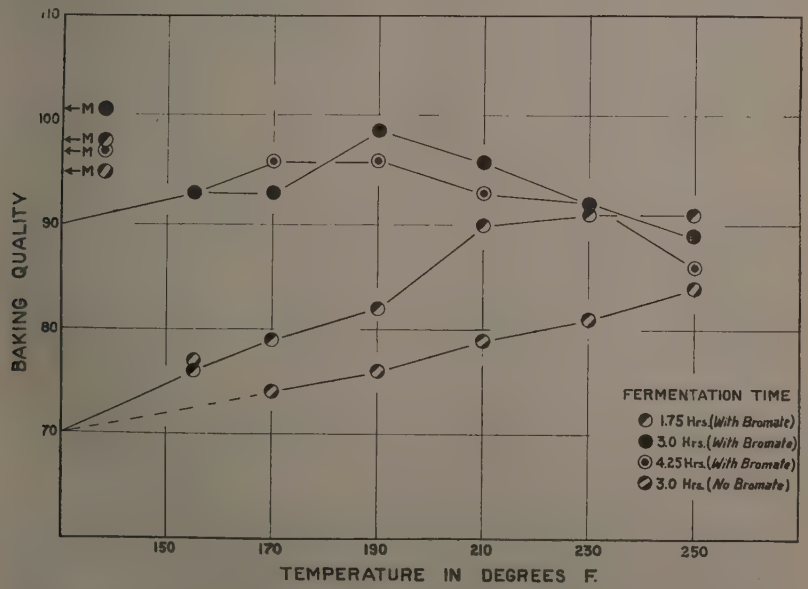


FIG. 2. *Influence of increasing temperature of heat treatment for 3 hr. on reducing the deleterious effect of germ on baking quality, (single figure estimate) of fifth middlings—5% germ mixtures (time of heating 3 hr.).*

TABLE VII

INFLUENCE OF HEATING GERM AT VARYING TEMPERATURES FOR THREE HOURS ON FERMENTATION TOLERANCE OF MIDDLINGS + 5% GERM MIXTURES

Sample	Treatment		Loaf volume, in cc.	General appearance	Crust color	Grain and texture	Single figure estimate
	Temperature, in ° F.	Time, in hr.					
Fermentation Time 3.00 hr., Basic Formula							
5th Middlings	Control		608	4.1 g	4.8 P	8.3 C&o	95
5th Middlings+5% raw germ			562	2.3 g	4.6 D	2.7 C&o	70
5th Middlings+5% germ	155	3	595	2.6 g	4.5 D	3.5 C&o	77
5th Middlings+5% germ	170	3	550	2.3 g	4.6 D	4.0	72
5th Middlings+5% germ	190	3	554	2.5 g	4.8 D	4.5	76
5th Middlings+5% germ	210	3	563	3.2 g	4.8 D	5.0	79
5th Middlings+5% germ	230	3	560	3.9 g	4.5 D	5.8	82
5th Middlings+5% germ	250	3	563	3.9 g	4.5 D	6.5	84
Fermentation Time 1.75 hr., Basic Formula +0.001% Pot. Bromate							
5th Middlings	Control		616	4.8	4.5 D	9.1	98
5th Middlings+5% raw germ			537	2.3 g	4.0 D	3.3	70
5th Middlings+5% germ	155	3	556	2.6 g	4.0 D	4.5	76
5th Middlings+5% germ	170	3	573	2.9 g	4.0 D	5.0	79
5th Middlings+5% germ	190	3	578	3.4 g	4.0 D	5.7	82
5th Middlings+5% germ	210	3	596	4.1 g	4.0 D	7.3	90
5th Middlings+5% germ	230	3	604	4.3 g	4.1 D	7.4	91
5th Middlings+5% germ	250	3	584	4.2 g	4.3 D	8.1	91
Fermentation Time 3 hr., Basic Formula +0.001% Pot. Bromate							
5th Middlings	Control		627	5.0	5.0	9.3	101
5th Middlings+5% raw germ			632	3.2 g	4.5 D	6.5 C&o	90
5th Middlings+5% germ	155	3	643	3.9 g	4.5 D	6.7 C&o	93
5th Middlings+5% germ	170	3	617	4.1 g	4.5 D	7.5	94
5th Middlings+5% germ	190	3	646	4.6 g	4.5 D	8.3	99
5th Middlings+5% germ	210	3	623	4.1 O	4.5 D	8.3	96
5th Middlings+5% germ	230	3	597	4.1 O	4.5 D	8.0	92
5th Middlings+5% germ	250	3	570	3.9 O	4.5 D	8.0	89
Fermentation Time 4.25 hr., Basic Formula +0.001% Pot. Bromate							
5th Middlings	Control		597	4.3 O	4.6 P	9.4	97
5th Middlings+5% raw germ			643	4.6 O	4.8 D	5.6	90
5th Middlings+5% germ	155	3	640	4.4 O	4.8 D	6.5	93
5th Middlings+5% germ	170	3	637	4.1 O	4.3 D	7.9	96
5th Middlings+5% germ	190	3	629	4.3 O	4.5 D	8.1	96
5th Middlings+5% germ	210	3	603	4.0 O	4.9 P	7.9	93
5th Middlings+5% germ	230	3	583	3.9 O	4.9 P	8.3	92
5th Middlings+5% germ	250	3	555	3.5 O	4.9 P	7.5	86

TABLE VIII

SUMMARY OF BAKING RESULTS
INFLUENCE OF HEATING GERM AT VARYING TEMPERATURES FOR THREE HOURS ON THE FERMENTATION TOLERANCE OF MIDDLINGS+5% GERM MIXTURES

Sample	Treatment		Loaf volume in cc.					
	Temperature, in °F.	Time in hr.	Basic procedure	Basic procedure + 0.001% $KBrO_3$	Reaction to bromate	Fermentation tolerance		
						1.75 hr.	3.0 hr.	4.25 hr.
5th Middlings	Control		608	627	+19	616	627	597
5th Middlings+5% raw germ			562	632	+70	537	632	643
5th Middlings+5% germ	115	3	595	643	+48	556	643	640
5th Middlings+5% germ	170	3	550	617	+87	573	617	637
5th Middlings+5% germ	190	3	554	646	+92	578	646	629
5th Middlings+5% germ	210	3	563	623	+60	596	623	603
5th Middlings+5% germ	230	3	560	597	+30	604	597	583
5th Middlings+5% germ	250	3	563	570	+ 7	584	570	555
Single Figure Estimate								
5th Middlings	Control		95	101	+ 6	98	101	97
5th Middlings+5% raw germ			70	90	+20	70	90	90
5th Middlings+5% germ	155	3	77	93	+16	76	93	93
5th Middlings+5% germ	170	3	74	93	+17	79	89	96
5th Middlings+5% germ	190	3	76	99	+23	82	99	96
5th Middlings+5% germ	210	3	79	96	+17	90	96	93
5th Middlings+5% germ	230	3	82	92	+10	91	92	92
5th Middlings+5% germ	250	3	84	89	+ 5	91	89	86

The baking results again show the remarkable influence of heat and bromate on reducing the deleterious effects of the germ on baking quality. The characteristics of the loaves are illustrated in Plates V and VI and the baking quality graphically represented in Fig. 2.

When baked by the basic procedure, the addition of "raw" germ resulted in a loaf possessing pronounced characteristics of underfermentation, namely glossy sides, flat top, sharp corners, lack of break and shred and small volume.

Increasing the fermentation time, heating the germ before admixture, or the addition of bromate each results in an improvement in baking quality as reflected in better handling qualities of the dough, loaf volume, crust characteristics, shape of loaf, break and shred, and in crumb texture. When these different methods of reducing the deleterious effects of the germ were combined the maximum baking quality was obtained by heat treatment of the germ at 190° F. and baking with the addition of 0.001% potassium bromate, using a three-hour fermentation. With a fermentation time of 4.25 hr. the loaves baked by the bromate method all had a slightly over-fermented appearance, this characteristic becoming progressively more pronounced, as the temperature to which the germ had been heated increased. This is evidence that the changes induced in the germ may be carried too far to secure the maximum baking quality and indicates that over treatment reduces the fermentation tolerance.

The relation between heat treatment of germ and the response of the flour-germ mixtures to the addition of potassium bromate to the baking formula is very marked. All the mixtures are of similar protein content but the response to bromate varies, depending on the heat treatment the germ received—the more severe the heat treatment the less the response to bromate. Evidently heat treatment and bromate produce similar effects in so far as their influence on baking quality is concerned: this leads to the suggestion that the improving effect of heat treatment is linked up with the oxidation of certain germ constituents—presumably the phosphatides.

Physico-Chemical and Chemical Changes Induced by Heating Germ

A study of physico-chemical and chemical changes induced by heating germ is being made and will be reported in a subsequent paper. However, the hydrogen ion concentration of a few germ extracts has been determined by the A.A.C.C. method [1] as outlined for flour, using the quinhydrone gold electrode and the apparatus described in a previous paper [4]. Two samples have also been submitted to ether extraction and the iodine number of the ether-free extract determined by Wij's method.

The results are recorded in Table IX.

TABLE IX

INFLUENCE OF HEATING GERM ON HYDROGEN ION CONCENTRATION AND IODINE NUMBER

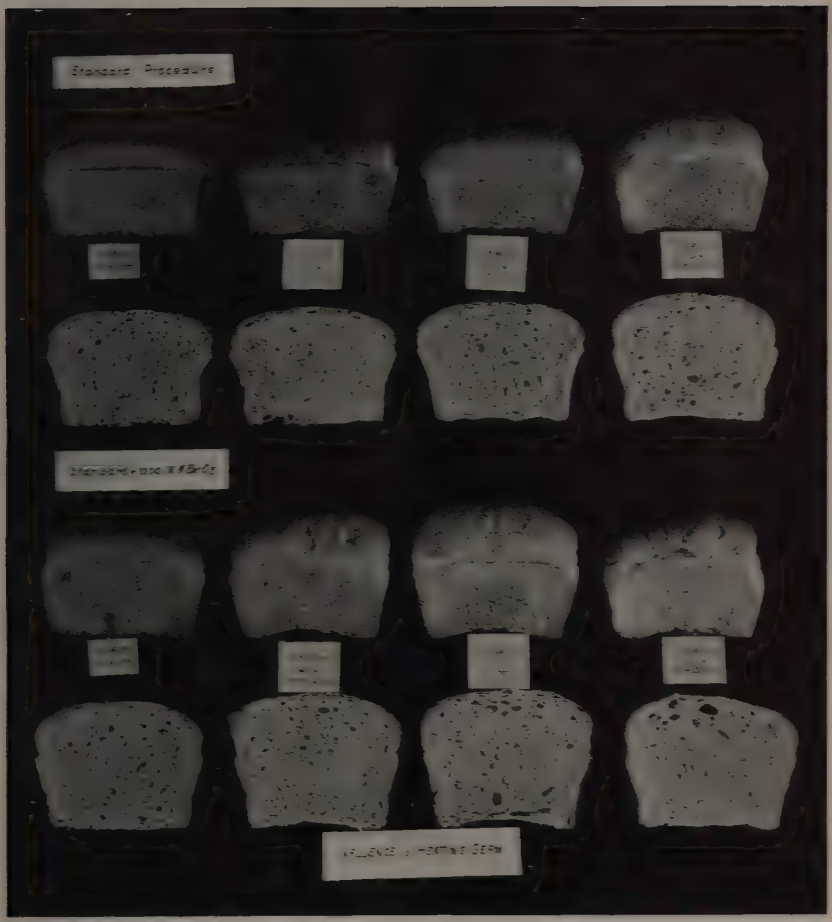
	pH	Iodine No. (Wij's)
"Raw" germ	6.51	125.6
Germ heated at 190° F. for 3 hr.	6.27	
Germ heated at 250° F. for 2 hr.	5.94	111.5

These preliminary experiments reveal a marked increase in the H-ion concentration of the aqueous extract of the germ and a lower iodine number of the ether extract (which may be due to oxidation).

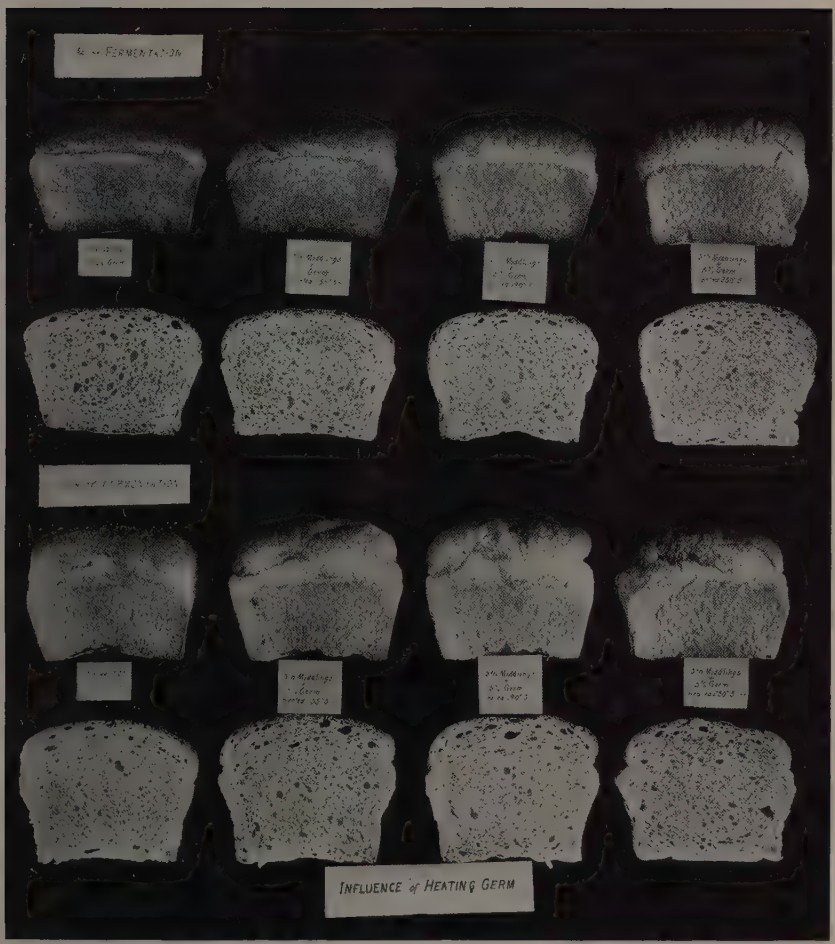
Discussion

The experiments reported in this paper seem to offer convincing evidence that the improvement in baking quality induced by the heat treatment of straight-grade flour is to be ascribed to changes in the germ constituents. The heat treatment of an untreated and unaged highly refined flour stream resulted in no essential improvement in baking quality; nor did heat treatment of ether-extracted straight-grade flour. Flours which gave little or no improvement on heat treatment also showed no essential responses on addition of potassium bromate to the baking formula.

The addition of untreated germ to middlings flour resulted in a marked degradation in baking quality as determined without bromate. This was reflected in poorer handling qualities of the dough, a very "green" appearance



The influence of heating germ at varying temperatures expressed in °F. on the baking quality of flour—germ mixtures determined by the loaf procedure.



The influence of heating germ at varying temperatures on the baking quality (determined with bromate) of flour—germ mixtures.

of the baking loaf, a decreased volume and a coarse, open crumb texture. These harmful effects cannot be ascribed to proteases added with germ since the detrimental effects progressively decreased with an extension of the fermentation time. The addition of bromate or the heating of the germ markedly reduced its detrimental influence, and at the same time, reduced the fermentation period necessary to produce a loaf possessing the characteristics of normal fermentation. As the severity of the heat treatment of the germ was increased the response on addition of bromate to the baking formula became progressively less. Since these germ-middlings mixtures were of similar protein content these results indicate that the response of a natural flour to bromate depends in part at least, on the state of oxidation, assuming that this is the reaction involved, of certain germ constituents.

These preliminary experiments, while far from complete, serve to call attention to the importance of germ in flour, and suggest a method of attack for investigations on the aging of flour, and the action of oxidizing agents, used as chemical improvers—a field which has already been opened up by Working.

It is well known that the fermentation tolerance, the reaction to bromate, and the improvement incidental to aging is greater, the lower the grade of flour, that is, in those flours which are undoubtedly more contaminated with germ. This higher fermentation tolerance and the greater reaction to bromate has been commonly ascribed to the higher protein content of the lower grades, but the experiments reported here suggest that the prime factor responsible for these differences is the greater proportion of germ. Indirect evidence points to the phosphatides of the germ as the constituents involved in these reactions. Working has pointed out the ease with which they undergo oxidation, and in the present study, it has been shown that the addition of lecithin to a highly refined flour causes an enormous response in loaf volume on the addition of bromate to the baking formula. It is suggested that the action of bromate is primarily on the labile phosphatides producing changes which decrease the detrimental effect of these substances on the gluten, thus allowing the inherent characteristics of the gluten to come into play. It would thus be expected that the higher the gluten content the greater would be the response to bromate. The action of bromate, however, does not seem to be confined to the germ constituents since overheated fifth middlings flour exhibited a negative reaction to bromate. Over-oxidation thus appears to be detrimental to baking quality, and it is interesting to note that the addition of "raw" germ improves the baking quality of over-heated fifth middlings flour. This result may probably be due to the unoxidized phosphatides of the unheated germ acting as a reducing agent, thus tending to reduce the harmful effects of over-oxidation of the endosperm constituents.

This is the converse of the experiments of Kent-Jones, who reported an improvement in the baking quality of natural flour, when less than 1.0% of highly over-heated flour was added. If the writer's theory is tenable, there may be a sound theoretical basis for this claim. The oxidized phosphatide in the heated flour may serve to oxidize, or act as a catalyst in the oxidation of

the phosphatide present in the reduced form in the natural flour. On the other hand, the phosphatides of the unheated flour may be oxidized by the over-heated endosperm constituents.

Whether or not these premises are valid, must await the results of further investigation, but the results of these preliminary experiments indicate that the germ constituents may play an important rôle in the baking quality of natural flours; they explain the baking results obtained by heat treatment. The improvement in baking quality observed by the heat treatment of natural flour can be simulated by heat treatment of the germ alone. Heat and bromate both produced the same general effects on the germ, the higher the temperature to which the germ was heated the lower was the response of the germ-flour mixtures to bromate (which is similar to the behavior of the heat treated straight-grade flours). Heat treatment of straight-grade flour resulted in less enhancement than that induced by chemical improvers such as agene and potassium bromate. The explanation lies in the simultaneous production of both desirable and undesirable changes by heat. The biochemical studies reported in a previous paper revealed that heat treatment caused damage to gluten quality by every measure used for its determination, even in the case of those samples which showed a distinct improvement in baking quality when determined by the basic procedure. The explanation lies in the beneficial effect of heat on the germ constituents in so far as their influence on baking quality is concerned. Due to the decreased gluten quality, the improvement is less than that brought about by the use of chemical improvers which appear to act primarily on the germ constituents.

The suggestion of Kent-Jones that the improvement which he observed in the baking quality of natural flour by the addition of 0.7% overheated flour was due to a greatly increased imbibitional capacity of the gluten proteins of the overheated flour is untenable from theoretical and practical considerations. An increased imbibitional capacity on extensive heat coagulation is contrary to the experimental observations of others. Assuming that such did occur, it is inconceivable that the gluten contained in 0.7 gm. of overheated flour when dispersed in 99.3 gm. natural flour would serve to explain the improvement in the handling qualities of the dough as a whole.

The conception concerning the rôle of the germ constituents advanced by the writer has an important bearing on the problem of changes in flour incidental to aging. If the writer's premises are valid, the improvement in baking quality due to aging is primarily associated with changes in certain germ constituents—presumably the phosphatides. It is suggested that straight-grade flours and the low grade mill streams show greater improvement on aging than the high grade flours, owing to their being more highly contaminated with germ. An investigation on the rôle of germ constituents in aging of flour is now under way and the results will be reported later.

Summarizing, the influence of heat as reflected in baking quality is detrimental to the gluten, but markedly decreases the deleterious effects of the germ constituents. Hence unaged flours containing low grade mill streams may show considerable improvement due to proper heat treatment despite the injury to gluten quality, when baked without chemical improvers.

Acknowledgments

The author wishes to thank Dr. C. H. Bailey of the University of Minnesota, for his counsel and advice, and Mr. A. W. Alcock for his kindness in judging the baked loaves and his assistance in the bleaching experiments. He is also indebted to Mr. S. T. Hadley for valuable technical assistance.

References

1. American Association of Cereal Chemists. Methods for the analysis of cereals and cereal products. Lancaster Press Inc. Lancaster, Pa. 1928.
2. Berliner, E. and Ruter, R. Z. ges. Muhlenwesen, p. 209. 1928.
3. Geddes, W. F. Can. J. Research, 1:528. 1929.
4. Geddes, W. F. Can. J. Research, 2:65. 1930.
5. Johnson, A. H. Cereal Chem. 5:169. 1928.
6. Kent-Jones, D. W. Cereal Chem. 5:235. 1928.
7. Winton, A. L. U.S. Dept. Agr. Bur. Chem. Bul. 137:144. 1911.
8. Working, E. B. Cereal Chem. 1:153. 1924.
9. Working, E. B. Cereal Chem. 5:223. 1928.
10. Working. E. B. Cereal Chem. 5:431. 1928.

STUDIES OF REACTIONS RELATING TO CARBOHYDRATES AND POLYSACCHARIDES.

XXVIII. THE STRUCTURE OF ISOPROPYLIDENE GLYCEROL[1]

By Harold Hibbert[2] and J. G. Morazain[3]

Abstract

Isopropylidene glycerol, prepared in neutral medium by the action of anhydrous copper sulphate as condensing agent, was methylated with silver oxide and methyl iodide; the product yielded only glycerol α-methyl ether on hydrolysis, thus proving the absence of any six-membered ketal in the condensation product of glycerol and acetone.

The properties of both glycerol α- and β-methyl ethers have been carefully redetermined.

A table of the isomeric acetals and ketals summarising their physico-chemical constants is given, in view of their usefulness as "type compounds" in investigations relating to fats, carbohydrates and polysaccharides.

In a previous communication (5) it was shown that in the condensation of acetone and glycerol in a neutral medium, only one of the two possible ring forms was produced, namely the five-membered isopropylidene glycerol.

This was proved by methylation of the above-mentioned ketal, using dimethyl sulphate and a 30% solution of sodium hydroxide.

The methyl ether thus obtained was then hydrolysed; it yielded only glycerol α-methyl ether, thus proving the absence of the six-membered ketal.

A milder type of methylation appeared advisable, however, due to the large amount of hydrolysis occurring during the above-mentioned methylation process,—with the possibility, under such conditions, of ring scission of the six-membered derivative. For this reason, it seemed desirable to substitute the Gilchrist and Purves method of methylation (4). It was found that when the isopropylidene glycerol (prepared in neutral medium) was methylated with silver oxide and methyl iodide, isopropylidene glycerol α-methyl ether was obtained in 76% yield. This, on hydrolysis, yielded only glycerol α-methyl ether, again indicating the absence of the six-membered isomer. *It would thus seem to be definitely established that in the condensation of acetone and glycerol in either neutral or acid medium only the five-membered ketal is formed.*

As the use of glycerol α- and β-methyl ethers as "type substances" is finding considerable application in the determination of the structure of fats (1, 2, 3), it seemed advisable to make careful determinations or re-determinations of their physical constants at various temperatures; these are tabulated on next page.

[1] *Manuscript received February 5, 1930.*

Contribution from the Industrial and Cellulose Chemistry Laboratories, McGill University, Montreal, Canada.

[2] *Professor of Industrial and Cellulose Chemistry, McGill University.*

[3] *Post-graduate student, McGill University, and holder of a Studentship under the National Research Council of Canada.*

TABLE I

PROPERTIES OF GLYCEROL METHYL ETHERS

Constants	Glycerol α-methyl ether	Glycerol β-methyl ether
Boiling point	110°/13 mm. 221°/754 mm.	122-123°/13 mm.
Refractive index	at 25°: 1.4430 at 20°: 1.4450 at 12°: 1.4472	at 20°: 1.4485 at 17°: 1.4494 at 14°: 1.4501
Density (D_{4°)	at 22°: 1.1169 at 17°: 1.1197	at 22°: 1.1273 at 18°: 1.1293

The physico-chemical constants of the important cyclic acetals and ketals are summarized in the following table:

Experimental Details

Methylation

44 gm. (one mol) of isopropylidene glycerol (prepared as previously described by the use of anhydrous copper sulphate at room temperature (5), was methylated with 107 gm.of silver oxide and 230 gm. of methyl iodide (five mols of each). This was accomplished by dissolving the ketal in the methyl iodide, in a three-neck flask fitted with a stirrer and a condenser, heating to 40° C., and adding the silver oxide in ten equal lots, one lot every 20 minutes. The heating at 40° C. was continued for one hour after the addition of the last lot, and the reaction mixture then extracted five times with hot chloroform, and the residue finally washed with the hot solvent on the filter. Evaporation of the chloroform and fractionation yielded 33 gm. (76%) of isopropylideneglycerol α-methyl ether, b.p. 58-60°/14 mm.; 154°/774 mm.; $^{n}D^{20}$: 1.4150.

Hydrolysis

22 gm. of the isopropylidene glycerol α-methyl ether thus obtained was then hydrolysed by heating at 60° C. with about twice its volume of water containing one drop of concentrated hydrochloric acid. After one hour the reaction mixture was cooled, neutralised by shaking first with lead carbonate and then with silver carbonate, and the solution filtered. The water was removed from the solution by distillation under reduced pressure (30 mm.), and the residual oil consisting of glycerol α-methyl ether then distilled at 13 mm. pressure. After removal of the small amount of water present, the glycerol α-methyl ether distilled over very constantly at 110°/13 mm., $^{n}D^{24}$:1.4432. There was no indication of the presence of any of the isomeric glycerol β-methyl ether, b.p. 122-123°/13 mm., as indicated by the boiling point, refractive index and the melting point of the di-*p*-nitrobenzoate.

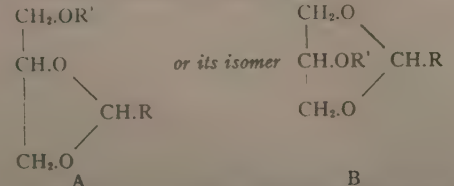
The use of isopropylidene glycerol by Averill, Roche and King (1), and other workers, for the determination of the structure of fats is thus warranted.

TABLE II

PHYSICO-CHEMICAL CONSTANTS OF CYCLIC ACETALS AND KETALS

I Nature of acetal or ketal	II Formaldehyde	III Acetaldehyde	IV Benzaldehyde	V p-nitro-benzaldehyde	VI Cinnamic Aldehyde	VII Chloral	VIII Acetone
Type A with $R' = CH_3$	Bp. 147°/760 mm. $^nD^{20}$: 1.4213 d_4^{20} : 1.0788 (1)	Bp. 56-58°/23 mm. $^nD^{17}$: 1.4177 d_4^{17} : 1.0224 (2)	Bp. 138-145°/10 mm. (3)	Mp. 47° and 42° (4)	Bp. 164-166°/6 mm. (5)	Bp. 108°/10 mm. $^nD^{20}$: 1.4806 d_4^{20} : 1.4228 (6)	Bp. 57°/14 mm. 154°/774 mm. $^nD^{20}$: 1.4150 (7)
Type B with $R' = CH_3$	Bp. 152°/760 mm. $^nD^{20}$: 1.4295 (1)	Bp. 80°/23 mm. $^nD^{17}$: 1.4375 d_4^{17} : 1.0705 (2)	Mp. 52° (3)	Mp. 139° & 106° (4)	Mp. 79-80° (5)		
Type A with $R' = C_6H_5CO$	Bp. 172-175°/15 mm. (1)	Bp. 144-145°/2 mm. $^nD^{17}$: 1.5145 d_4^{17} : 1.1618 (2)		Mp. 178° & 115° (4)		Mp. 73° & 81° (6)	Mp. 42° (7)
Type B with $R' = C_6H_5CO$	Mp. 72° (1)	Mp. 86° (2)	Mp. 103° (3)	Mp. 204° & 159° (4)			
Type A with $R' = p\text{-}NO_2.C_6H_4.CO$				Mp. 117-118° & 110° (4)		Mp. 102° & 102.5° (6)	Mp. 56° (7)
Type B with $R' = p\text{-}.NO_2\,C_6H_4\,CO$				Mp. 208° & 202°			

NOTE:—*The acetal or ketal is in every case of the type*



The substituent R' being given in Column I and the substituent R.CH(O) at the head of the remaining columns. In these columns (2, 3, 4, 5, 6, 7 and 8) respectively are given the physical properties of the cyclic acetal or ketal corresponding to the type substance shown in Column I.

(1) *H. Hibbert and N. M. Carter. J. Am. Chem. Soc. 50:3120-3127. 1928. p. 3124.*

(2) *H. S. Hill, A. C. Hill and H. Hibbert. J. Amer. Chem. Soc. 50:2242-2249. 1928. p. 2246.*

(3) *H. S. Hill, M. S. Whelen and H. Hibbert. J. Am. Chem. Soc. 50: 2235-2242. 1928. p. 2240.*

(4) *H. Hibbert and N. M. Carter. J. Am. Chem. Soc. 50:3376-3388, 1928. p. 3379.*

(5) *H. Hibbert and M. S. Whelen. J. Am. Chem. Soc. 51:620-625. 1929. p. 624.*

(6) *H. Hibbert, J. G. Morazain and A. Paquet. Can. J. Research, 2: 1930.*

(7) *H. Hibbert, and J. G. Morazain. Can. J. Research, 2:35-41, 1930.*

References

1. Averill, H. P., Roche, J. N. and King, C. G. J. Am. Chem. Soc. 51:866-872. 1929.
2. Fairbourne, A. and Cowdrey, G. W. J. Chem. Soc. 129-135. 1929.
3. Fairbourne, A. J. Chem. Soc. 1151-1153. 1929.
4. Gilchrist, H. S. and Purves, C. B. J. Chem. Soc. 127:2735-2745. 1925.
5. Hibbert, H. and Morazain, J. G. Can. J. Research 2:35-41. 1930.

NOTE ON THE ISOMERIC NITROBENZYLIDENE GLYCEROLS[1]

By Harold Hibbert[2]

It is necessary to call attention to the recent article by Tanasescu and Macovski (2) on "Photochemical Reactions of o-nitrobenzylidene glycerol and its derivatives".

These authors state that the researches of Hibbert and co-workers (1) show that only one isomer, namely, the five-membered acetal, is present in the reaction product formed by the condensation of p-nitrobenzaldehyde, benzaldehyde, etc. with glycerol, which they claim is in agreement with their own work on the condensation of o- and m-nitrobenzaldehydes.

Such a statement appears quite incomprehensible in view of the fact that the object of the researches (1) quoted by Tanasescu and Macovski was the isolation of *both* the five- and six-membered glycerol cyclic acetals, a task which was successfully accomplished.

In other words, it was demonstrated for the first time beyond question by Hibbert and co-workers in a series of researches (1) on the condensation of formaldehyde, acetaldehyde, benzaldehyde and p-nitrobenzaldehyde with glycerol, that in all of these cases a mixture of the isomeric five- and six-mem-membered glycerol cyclic acetals is formed.

That Tanasescu and Macovski were unable to isolate similar isomers in the case of o- and m-nitrobenzaldehyde is presumably due to their unfamiliarity with the subject and the necessary technique.

References

1. Hibbert, H. and co-workers. J. Am. Chem. Soc. 50: 2235, 2242, 3376. 1928. See also 50: 3120. 1928.
2. Tanasescu, I. and Macovski, E. Bull Soc. chim. (4), 45: 1022-1030. (1929.

[1] *Manuscript received March 8, 1930.*

[2] *Professor of Industrial and Cellulose Chemistry, McGill University.*

FREEZING POINTS AND HEAT CAPACITIES OF AQUEOUS SOLUTIONS OF POTASSIUM CHLORIDE[1]

By W. H. Barnes[2] and O. Maass[3]

Abstract

Freezing points for the system $KCl-H_2O$ are determined to 0.1° and 0.1% concentration over a range of concentrations including the eutectic. The solubility of potassium chloride in water at 25.22° C. is measured. The heat capacities of a few rapidly and slowly frozen aqueous solutions of potassium chloride are investigated. The heat capacities of an approximately eutectic aqueous solution of potassium chloride are studied.

Supercooling of aqueous solutions of potassium chloride is observed to be a constant factor under fixed cooling conditions, and its magnitude depends on the concentration of the solution. Aqueous solutions of potassium chloride, cooled rapidly to temperatures below the eutectic, are found to have approximately the same heat capacity values as those cooled slowly to the same temperatures. The heat of solution of potassium chloride at −10.7° C. to form the eutectic concentration is calculated, and the relation between the phase rule diagram and heat capacities is discussed.

I. General Introduction

This paper contains the results of a number of heat capacity measurements on aqueous solutions of potassium chloride which, together with some theoretical considerations, lead to a calculation of the heat of solution of KCl to form the eutectic concentration at −10.7° C. In connection with this work, the freezing point curve for the system $KCl-H_2O$ was redetermined, and the results are included below. Finally the heat capacities of some rapidly and slowly frozen aqueous solutions of KCl were undertaken for reasons which will appear later, and the conclusions to be drawn from such experiments are indicated.

The paper is divided into several parts, Sections II, III, and IV, each complete in itself in so far as experimental work is concerned. Finally, in the General Discussion (Section V), the interdependence of the different parts is shown, and various points of interest are emphasized.

II. Freezing Point Curve for the System $KCl-H_2O$

Introduction

For the heat capacity measurements described later, it was necessary to have reliable data on the freezing points of aqueous solutions of potassium chloride. A survey of the literature (9), gave such discordant information that a redetermination was made of this freezing point curve. Values for the

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Contribution from the Physical Chemistry Laboratory of McGill University, Montreal. Part of the work outlined (Sections II and IV) was undertaken while one of the authors (W.H.B.) held a Fellowship under the National Research Council.

[2] *Sessional lecturer in Physics, McGill University.*

[3] *Professor of Physical Chemistry, McGill University.*

temperature and concentration of the eutectic respectively have been obtained by different investigators as follows: Meusser (13) −9.0° C., 19.3%; de Coppet (6) −11.1° C., 19.8%; Ponsot (14) −10.64° C., 19.5%; Rodebusch (16) −10.66° C.

After the present investigation was completed, values for the $KCl-H_2O$ freezing point curve appeared in the International Critical Tables (7) in terms of the molal freezing point lowering per number of gram formula weights per 1,000 gm. of solution. Taking the molecular weight of KCl as 74.56 these figures have been converted into the nomenclature of the present paper, namely, grams of potassium chloride per 100 gm. of solution, and are given later in Table II. The I.C.T. values for the freezing point and concentration of the eutectic are −10.69° C. and 19.74% respectively.

Experimental Work

Kahlbaum's potassium chloride was recrystallized several times from distilled water, and finally was kept at a dull red heat in a platinum crucible for two or three hours in order to drive off all moisture.

Four solutions of different concentrations of potassium chloride were made up in the following manner. Calibrated standard flasks of 50 cc. capacity were thoroughly cleaned and then dried at 110° C. Required amounts of dry potassium chloride were weighed directly in the flasks, and the solutions made were brought to the graduation marks at a temperature of 20–21° C. All weighings were corrected to standard brass weights in vacuo. From density data given by Landolt-Bornstein (9, p. 401) a curve of grams of potassium chloride per 100 cc. of solution was plotted against grams of potassium chloride per 100 gm. of solution, and the per cent concentrations of the solutions were determined from the curve.

Finally, three solutions of approximately 19% concentration were made up directly by weighing the potassium chloride, adding the required amounts of freshly boiled distilled water, and weighing the solutions. All weights were corrected to brass weights in vacuo.

The freezing point apparatus employed was a modification of the usual Beckmann arrangement. A small test-tube containing the solution was held in the centre of a larger tube by means of a cork. The space between the tubes was filled with air. The resulting double-walled tube was suspended in a Dewar flask full of ether. The temperature of the ether was regulated by the addition of small pieces of solid carbon dioxide by hand. This bath was stirred by bubbling a stream of dry air through the ether. Temperatures of the potassium chloride solutions were obtained from a calibrated mercury thermometer graduated in tenths of a degree. The thermometer passed through the centre of a glass stirrer which was made to move vertically in the inner test-tube by means of an electrically driven eccentric.

The temperature of the solutions was allowed to fall slowly at a rate of less than 1° per minute until crystallization commenced. The ether bath was removed, and the temperature of the solutions allowed to rise at a rate of about 1° per five minutes.

In all cases, with the exception of two of the approximately 19% solutions, the temperatures at which the first particle of ice appeared on cooling, and the last particle disappeared on warming the solutions were recorded.

At least two samples of each solution were tested and several experiments were made on each sample.

The observations given in Table I are typical, and show the agreement among the several readings taken. With all the solutions, this agreement is better for the temperature (T_2) at which the last particle disappeared on warming, than for the temperature (T_1) at which the first particle appeared on cooling.

TABLE I

FREEZING AND MELTING POINTS OF AQUEOUS POTASSIUM CHLORIDE SOLUTIONS*

Sample	Experiment	Temperatures (uncorrected)	
		T_1	T_2
A	1	−4.71	−4.10
	2	−4.69	−4.11
B	1	−4.50	−4.11
	2	−4.44	−4.10

**Composition of solution 9.48%.*

The temperature of the eutectic was obtained by making three sets of time-temperature observations on a 19.02% solution of potassium chloride. The concentration at the eutectic was found by reading the intersection of the freezing point curve with the ordinate corresponding to the value found for the eutectic temperature.

Finally, two solubility determinations of potassium chloride in water at a temperature of about 25° C. were carried out by the method developed by Richards and Yngve (15) for sodium sulphate.

Results

The results obtained with various concentrations of potassium chloride, and used to plot the freezing point curve, are given in Table II. The first column gives the concentration of the solution in grams of potassium chloride per 100 gm. of solution. In the second and third columns are recorded the temperatures of the appearance of the first particle of ice on cooling (T_1), and of the disappearance of the last particle on warming (T_2), respectively. These values are the means of several determinations; the maximum deviation of any single observation from the mean was not greater than 0.15° C. for T_1, and 0.05° C. for T_2. Corrections are applied for errors in reading and for stem exposure. The fourth column contains the means of the values given in the two preceding columns for each concentration. Finally, in the fifth and sixth columns are shown the values given by the I.C.T. (International Critical Tables) for the concentrations and freezing points respectively.

TABLE II

FREEZING POINTS OF AQUEOUS POTASSIUM CHLORIDE SOLUTIONS OF VARIOUS CONCENTRATIONS

(This Paper)				(I.C.T.)	
Concentration (%)	T_1 (°C.)	T_2 (° C.)	T (° C.)	Concentration (%)	T (° C.)
4.95	− 2.34	− 2.13	− 2.24	0.741	−0.345
9.48	− 4.84	− 4.35	− 4.60	1.47	−0.678
13.70	− 7.11	− 6.65	− 6.88	3.60	−1.66
17.85	− 9.87	− 9.09	− 9.48	6.96	−3.25
18.94	—	− 9.78	—	10.06	−4.84
19.02	−10.68	− 9.93	−10.31	12.98	−6.44
19.49	—	−10.36	—	18.28	−9.69

The values given in Table II are plotted in Fig. 1 where C represents concentration of the solution and T temperature in degrees Centigrade. Points on the curve shown by double circles are values of T_1 and those shown by single circles are values of T_2. The dotted curve is plotted from the values of the I.C.T. recorded in Table II.

The three time-temperature determinations on the 19.02% solution gave values of −10.77° C., −10.70° C., and −10.70°C. respectively, with a mean of −10.72°C. for the temperature of the eutectic.

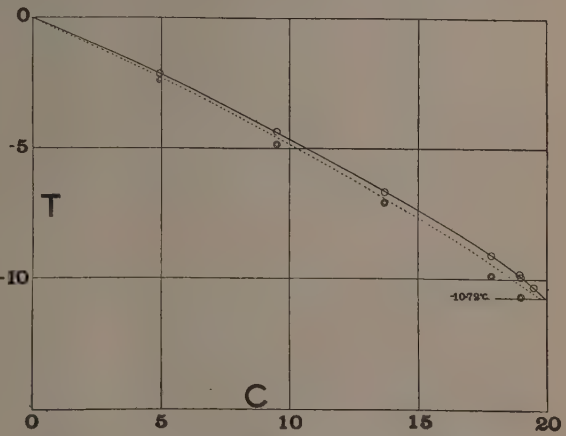

FIG. 1. *Relation of freezing point to concentration of aqueous potassium chloride solutions.*

The intersection of the curve through the temperatures (T_2) in Fig. 1 with the ordinate −10.72 gives 19.93 gm. potassium chloride per 100 gm. of solution as the concentration at the eutectic.

The solubility determinations were carried out in a thermostat at 25.22° C., the temperature of which was obtained from a calibrated Beckmann thermometer. The value found at this temperature was 26.41 ± 0.02 gm. potassium chloride per 100 gm. of solution.

III. Heat Capacities of Rapidly and Slowly Frozen Aqueous Salt Solutions

Introduction

In a paper published in 1916, McIntosh and Edson (12) described a series of interesting observations on colloidal solid solutions. They found that if an aqueous salt solution was frozen suddenly by immersion in liquid air or solid carbon dioxide, the resulting mass was microscopically homogeneous. No separation of ice and salt could be distinguished, and analyses of sections cut from various parts of the mass proved that the composition was uniform throughout.

If these rapidly frozen solutions contain salt and ice particles of colloidal dimensions in solid solution the fact might be capable of demonstration by measurements of heat capacities; in such an event, the surface per mol of salt will be very much greater than in a case of slow freezing, when the salt is deposited in a truly crystalline condition. Consequently, there should be a larger amount of energy associated with the surface, and this surface energy should appear as heat when solution of the salt takes place. In the case of potassium chloride, for which the heat of solution is negative, the numerical value of the heat of solution should be decreased. Hence, the heat absorbed when the frozen solution is allowed to warm up adiabatically from a known initial temperature to another known temperature above the melting point should be less in the case of a rapidly frozen solution than in the case of one that has been frozen slowly.

In order to test this point experimentally a few measurements of the heat capacities of a potassium chloride solution were made.

Experimental Work

The adiabatic calorimeter employed was that used at a later date for a determination of certain thermal constants of carbon dioxide, and is described elsewhere (10). For details with regard to this piece of apparatus and method of manipulation, reference should be made to the original description.

In the experiments with rapidly frozen solutions a platinum container, holding the solution and encased in a closed brass tube, was plunged into a mush of solid carbon dioxide and ether. For an initial temperature of $-78.5°$ C., it was allowed to remain in this mixture for about two hours before the container was transferred to the calorimeter. For higher initial temperatures it was kept in the carbon dioxide for about one hour, then transferred to a thermostat bath and kept at the desired temperature for another hour or two before the container was transferred to the calorimeter.

For the slow cooling experiments the brass tube was placed in a Dewar flask full of ether, and the temperature of the bath was reduced rapidly to 0° C. by the addition of solid carbon dioxide. From 0° C. to $-20°$ C. the temperature of the bath was reduced slowly at a rate of about ten degrees per hour by further additions of solid carbon dioxide. Finally, the container was removed to the thermostat and maintained at the desired initial temperature for another hour or two before being transferred to the calorimeter.

Mean values obtained for the heat capacities of a 12.95% solution of potassium chloride in calories per gram of solution from two different initial temperatures to +25.00° C. are given in the following Table III. This table also includes the results obtained for a rapidly frozen solution at a third temperature, because the value obtained is plotted on a curve to be described in the next section.

TABLE III

HEAT CAPACITIES OF AQUEOUS POTASSIUM CHLORIDE SOLUTIONS AND TYPE OF COOLING

Initial Temperature (°C.)	Type of Cooling	Number of Experiments	Mean heat capacity
−78.5	Rapid	9	128.86
−78.5	Slow	5	129.27
−37	Rapid	4	114.31
−37	Slow	5	114.83
−27	Rapid	4	110.28

The experiments described in this Section were carried out in the autumn of 1924 while developing the adiabatic calorimeter, and the accuracy attained at that time was not so high as in the subsequent work on carbon dioxide.

IV. Heat Capacities of Aqueous Solutions of Salts

Introduction

Parallel studies of the mechanism and heat quantities involved when a frozen aqueous salt solution is allowed to warm up adiabatically from an initial state of ice and salt at a given temperature to a final state of solution at another given temperature, lead to a number of interesting results and calculations.

Fig. 2 represents ideal diagrams for the heat capacity and freezing point curves. On the heat capacity curve, temperatures are plotted as abscissae, and ordinates give the heat capacities in calories per gram of solution from any given initial temperature to a fixed final temperature (T_1). The freezing point

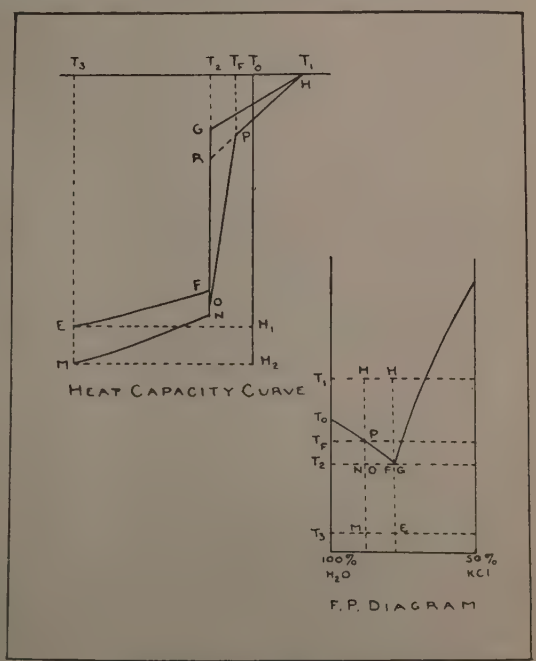

FIG. 2. *Heat capacity curve and freezing point diagram for the $KCl-H_2O$ system.*

diagram is shown in the usual manner with temperatures as ordinates and concentrations as abscissae. The letters on the heat capacity curves have the same significance as the corresponding letters on the freezing point diagram, and the same symbols for heat capacities and temperatures are employed in the equations derived below.

When one gram of a previously frozen aqueous solution of a salt of eutectic composition is allowed to warm up from an initial temperature (T_3) below the freezing point (T_2) to a final temperature (T_1), an amount of heat (H_1) is absorbed. The process is represented by EFGH on the heat capacity curve, and by the dotted line E(FG)H on the freezing point diagram. It consists of the following steps:—

1. Solid salt and ice warm up from T_3 to the temperature of the eutectic (T_2) taking up an amount of heat equal to $(m_1C_k+m_2C_i)\ (T_2-T_3)$, where C_k and C_i are the average specific heats, m_1 and m_2 the weights, of salt and ice respectively. Also $m_1+m_2=1$ since the heat capacities are expressed in calories per gram of solution. The curve EF of Fig. 2 represents this process graphically.

2. At the eutectic temperature the ice melts with an absorption of heat m_2L_2, where L_2 is the latent heat of fusion of ice in calories per gram at T_2.

3. The salt goes into solution at T_2 with an absorption of heat m_1S_2 where S_2 is the heat of solution per gram at temperature T_2 to form the eutectic concentration.

Operations 2 and 3 cause a halt (FG) on the freezing point diagram, and a decrease in the heat capacity represented by the ordinate FG at T_2. The numerical value of FG is thus equal to $m_2L_2+m_1S_2$.

4. Finally, the solution resulting from operation 3 warms up to T_1 with an absorption of heat equal to $(m_1+m_2)C_s(T_1-T_2)$, where C_s is the average specific heat of the solution. This is represented by the curve GH in Fig. 2.

The total heat measured is given by

$$H=(m_1C_k+m_2C_i)\ (T_2-T_3)+m_2L_2+m_1S_2+(m_1+m_2)C_s(T_1-T_2) \quad \ldots\ldots 1.$$

It is possible to consider several different mechanisms for transforming the ice and salt at T_3 to a solution at T_1, one of which is of interest. In this case the ice and salt may be imagined to warm up from the initial temperature (T_3) to 0° C. (T_o) at which temperature the ice melts, the temperature of the water and salt is then raised to the final temperature (T_1), where the salt goes into solution. The total heat (H_1) is the same as before but now is given by

$$H_1=m_1C_k(T_1-T_3)+m_2C_i(T_o-T_3)+m_2L_o+m_2C_w(T_1-T_o)+m_1S_1, \quad \ldots\ldots 2,$$

where C_w is the average specific heat of water, S_1 is the heat of solution of the salt per gram at T_1, and L_o is the latent heat of fusion of ice per gram at T_o.

In the case of a solution containing a lower concentration of salt than the eutectic, the ideal diagrams for the heat capacity and freezing point curves are represented in Fig. 2 by MNOPH.

Starting with m'_1 gm. of potassium chloride and m'_2 gm. of ice at temperature T_3, where $m'_1+m'_2=1$, and ending with one gram of solution at T_1, the mechanism is as follows:

1. The temperature of the ice and salt rises to T_2 (the eutectic). This is represented by the MN portion of the curves in Fig. 2.

2. At T_2 some ice melts, and salt goes into solution to form the eutectic composition, causing a halt (NO) on the freezing point diagram, and an absorption of heat (NO) on the heat capacity curve.

3. As further heat is taken up, the remainder of the ice melts, and the solution is diluted to the original concentration. The line OP on the heat capacity curve represents the integrated heats of solution, dilution, and fusion from T_2 to the freezing point (T_F).

4. Finally, the temperature of the solution rises from T_F to the final temperature T_1.

Since the total heat measured is independent of the intermediate steps by which the final state is reached, the heat capacity curve MNOPH can be replaced by MNRPH by allowing all the ice to melt at T_2, and then dissolving all the salt at the same temperature.

The difference in the heat capacities of the eutectic and the more dilute solution from T_2 to T_1 is equal to FN on the heat capacity curve. In the case of potassium chloride MN lies below EF because the heat capacity of ice is greater than that of potassium chloride and a larger proportion of the former is present in the more dilute solution.

Experimental Work

The heat capacities of a 19.80% solution of potassium chloride were determined with an improved type of adiabatic calorimeter the description of which will be given in a later paper. The results obtained from various initial temperatures to a final temperature of +25.00° C. are tabulated in Table IV, in which the initial temperature in degrees C. is given together with the heat capacity per gram of solution from the initial temperature to +25.00° C.

TABLE IV

HEAT CAPACITIES OF A 19.80% SOLUTION OF POTASSIUM CHLORIDE

Temperature	Heat Capacity
0.0	19.086
0.0	19.073
− 8.6	25.841
− 9.0	26.331
−14.7	100.93
−42.9	112.30
−44.0	112.79
−78.5	124.02
−78.5	124.15

The heat capacities given in Table IV are plotted (as circles) against initial temperature in Fig. 3 where H is heat capacity in calories per gram and T is initial temperature in degrees Centigrade. The dotted curve in this figure represents the values found in Section III for the 12.95% solution and recorded in Table II.

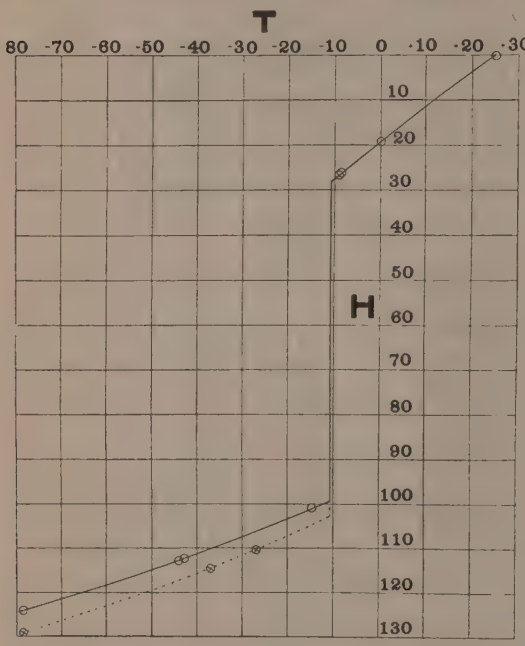

FIG. 3. *Relation of heat capacities of solutions of potassium chloride in water to the initial temperature. The values for the full line curve were obtained with a 19.80% solution; those for the dotted curve with a 12.95% solution.*

V. General Discussion

In Section II, the freezing point has been taken as the temperature at which the last particle of ice disappeared on allowing the frozen solutions to warm up slowly. This temperature was very constant for different experiments not only on the same sample of a given solution but on different samples. In view of this constancy and the slow rate of warming, it is probable that no appreciable lag in the thermometer was obtained.

The temperature at which ice first appeared in the solutions on cooling are of some interest, and have been included as points in Fig. 1.

It will be seen that, in general, supercooling given by the difference between the temperatures T_1 and T_2 increases with increasing concentration of the solution. Since the ability of the ice to crystallize readily probably is a function of an optimum relative orientation of ice molecules in the solution, it may be inferred that the presence of the molecules of potassium chloride would hinder the attainment of such orientation and thus give rise to supercooling, and that the effect would be more marked the higher the concentration of potassium chloride. Since the supercooling observed is so constant for any particular sample and increases so regularly with increasing concentration, it might be of interest to investigate this effect more thoroughly.

When several determinations were made at the same concentration the amount of supercooling was very constant, but showed a tendency to decrease slightly after the first freezing of the solution.

It may be noted that, although our freezing point curve lies above that given in the International Critical Tables, the means of the values of T_1 and T_2 at the different concentrations (listed as T in Column 4 of Table II) fall almost exactly on the I.C.T. curve.

A consideration of Table III, in Section III shows that the mean values for the slowly frozen solutions are about 0.5% higher than for those frozen rapidly. This is in accord with what would be expected if colloidal solid solutions result from the process of rapid freezing. On the other hand, an accuracy of only about 0.5% could be obtained with the apparatus at the time it was employed for these determinations, and deviations of individual experiments from the mean are greater than this in one or two cases. The results are of interest, however, in indicating that the effect sought for may be present, and in demonstrating that it must be less than 0.5% of the total heat capacity per gram.

In Section IV it has been shown that the difference in the total heats from the eutectic temperature to a fixed final temperature, for an eutectic solution and one of a lower concentration of salt, is equal to FN (Fig. 2). From Fig. 3 this difference between the 19.80% and the 12.95% solution is equal to 3.4 calories per gram. Hence the error introduced in using the heat capacities of the 19.80% solution for those of the eutectic composition found in Section II (19.93%) will be of the order of 0.07 calories at this temperature. As this is less than the experimental error involved in the heat capacity measurements, Equations 1 and 2 of Section IV can be applied to the values for the 19.80% solution as though it were of eutectic composition.

All the quantities on the right hand side of Equation 2 (Section IV) can be obtained from different sources in the literature. A calculation of the heat capacity per gram of a 19.80% solution of potassium chloride from −78.5° C. to 25.00° C. thus affords an interesting check on the value observed experimentally.

For the heat of solution (S_1) at 25.00° C. the data of Wust and Lange (17) at various concentrations was extrapolated to 19.80%. The molal heat of solution obtained was −3813 calories, which agrees with a recent experimental result obtained in this laboratory (4). From this value the heat of solution of 0.198 gm. of potassium chloride to form a 19.80% solution at 25.00° C. is equal to 10.12 calories (m_1S_1 in Equation 2).

Hence,

H_1 (calculated)

$$= m_1C_k(T_1-T_3)+m_2C_i(T_o-T_3)+m_2L_o+m_2C_w(T_1-T_o)+m_1S_1$$

$$= (0.198\times 0.160\times 103.5)+(0.802\times 0.431\times 78.5)+(0.802\times 79.42)$$

(3, 8) (11) (11)

$$+(0.802\times 1.002\times 25.00)+10.12$$

(2)

$$= 124.3 \text{ calories per gram of solution.}$$

and,

H_1 (observed)

$= 124.1$ calories per gram of solution.

The accuracy attained in the total heat measurements therefore is of the order of 0.15%.

Now from Equation 1 it is possible to calculate the heat of solution of potassium chloride at $-10.7°$ C. It will be seen that this equation includes the latent heat of fusion of ice at this temperature. From the Clausius equation (5), $(dL/dT) = C_w - C_i$, the rate of change of the latent heat of fusion with temperature (dL/dT) was calculated from the average specific heats of water (C_w) and of ice (C_i) respectively. The specific heat curve for water is known accurately to $-5°$ C. (1), and is perfectly smooth to above 25° C. Extrapolation to $-10.7°$ C. therefore can be made without appreciable error. In this way (dL/dT) between 0° C. and $-10.7°$ C. was calculated as 0.536 so that the latent heat of fusion of ice at $-10.7°$ C. is equal to 75.68 calories per gram.

The value of $(m_1+m_2)C_s(T_1-T_2)$ was obtained directly from Fig. 3 as 27.5 calories.

Hence,

$$H_1 = m_1C_k(T_1-T_3)+m_2C_i(T_2-T_3)+m_2L_2+m_1S_2+(m_1+m_2)C_s(T_1-T_2)$$

$$124.1 = (0.198\times0.158\times67.8)+(0.802\times0.426\times67.8)+(0.802\times73.68)$$

(11) (13)

$$+0.198S_2+27.5$$

Therefore,

$m_1S_2 = 12.2$ calories.

Thus the heat of solution of 0.198 gm. potassium chloride in 0.802 gm. of water at $-10.7°$ C. is equal to 12.2 calories. The molal heat of solution at this temperature to give this concentration, therefore, is equal to 4,600 calories.

By increasing the accuracy of the calorimetric measurements, and making use of total heats from an experimental curve rather than average values for specific heats, this method for determining the heats of solution at temperatures below zero should be capable of extended application. For instance, an accuracy of one part in 10,000 in the total heat measurements would increase the accuracy of the heat of solution value obtained above to one part in 1,000.

For eutectic solutions, heat capacity measurements can be obtained experimentally from initial temperatures of within one or two degrees below or above the eutectic temperature. For more dilute solutions, measurements are uncertain in the temperature region between that of the eutectic and the freezing point. Extrapolation of the "ice plus salt" and "solution" portions of the curve through this region, however, are possible. A consideration of Fig. 2 then shows that, given a heat capacity curve for a salt solution of any concentration, it is possible to determine the heats of solution at any temperature between 0° C. and the eutectic. For this purpose the latent heat of fusion of ice, at temperatures below zero, can be calculated with fair accuracy from existing data for water and ice, providing it is not necessary to extrapolate the specific heat curve for water too far below $-5°$ C.

It is of interest to note that in Equation 1 the sum of the first two terms on the right representing the heat capacity of the ice and salt from −78.5° C. to −10.7° C. is equal to 25.28 calories. The value obtained from the "ice plus salt" portion of the curve (Fig. 3) is equal to 25.1 calories.

From the heat capacity curve of Fig. 3 the average specific heat of a 19.80% solution of potassium chloride between −10° C. and +25° C. is 0.774 calories per gram per degree.

References

1. Barnes, H. T. and Cooke, H. L. Phys. Rev. 15:65. 1902.
2. Barnes, H. T. Trans. Roy .Soc. (A) 199:149-263. 1902.
3. Brönsted, J. N. Z. Elektrochem. 20: 554-556. 1914.
4. Buxton, K. S. Unpublished results.
5. Clausius, R. Mechanical Theory of Heat. I. p. 94.
6. de Coppet, L. C. Z. physik. Chem. 22:239-240. 1897.
7. International Critical Tables, (1928), Vol. 4. p. 259.
8. Koref, F. Ann. Physik. (4) 36:49-73. 1911.
9. Landolt-Börnstein Tab. (1923), Vol. 1, p. 626.
10. Maass, O. and Barnes, W. H. Proc. Roy Soc. (A)III:224-244. 1926.
11. Maass, O. and Waldbauer, L. J. J. Am. Chem. Soc. 47:1-9. 1925.
12. McIntosh, D. and Edson, R. J. Am. Chem. Soc. 38:613-615. 1916.
13. Meusser, A. Z. anorg. allgem. Chem. 44:79-80. 1905.
14. Ponsot, A. Compt. rend. 129:98-100. 1899.
15. Richards, T. W. and Yngve, V. J. Am. Chem. Soc. 40:164-174. 1918.
16. Rodebusch, W. H. J. Am. Chem. Soc. 40: 1204-1213. 1918.
17. Wüst, J. and Lange, E. Z. physik. Chem. 116:161-214. 1925.